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Metal stress modulates the immediate plasmid uptake potential of soil microbes

Klümper, Uli; Dechesne, Arnaud; Riber, Leise; Gülay, Arda; Brandt, K.K.; Sørensen, S.; Smets, Barth F.

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13th Symposium on

BAGECO 13

Bacterial Genetics and Ecology

THE MICROBIAL CONTINUITY ACROSS CHANGING ECOSYSTEMS

14–18 June 2015
Milan, Italy

PROGRAM

www.bageco2015.org

conventus
CONGRESSMANAGEMENT

Sun, 14 June 2015	Mon, 15 June 2015	Tue, 16 June 2015	Wed, 17 June 2015	Thu, 18 June 2015
	08:30–10:20	08:30–10:40	08:30–10:20	09:00–10:30
	Session I Microbial diversity and functioning in the soil ecosystem p. 15	**Session III** Symbiosis as a driving force of ecosystems II p. 17	**Session V** Gradients in conventional and extreme habitats p. 20	**Session VI continued** Novel biodegradation pathways along spatial gradients II p. 22
	Industrial Exhibition & Coffee Break			Coffee Break
	10:50–12:40	11:10–12:20	10:50–12:20	11:00–12:30
	Session II Microbial diversity and functioning in soil and non-conventional ecosystems p.15	**Session IV** The food to gut microbial continuity I p. 17	**Session VI** Novel biodegradation pathways along spatial gradients I p. 20	**Session VII** Constructed microbial communities as a tool in microbial ecology ecosystems p. 22
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	Session III Symbiosis as a driving force of ecosystems I p. 16	**Session V** The food to gut microbial continuity II p. 18	**Session VII** Adaption and the role of HGT p. 21	Farewell & announcements p. 23
		Industrial Exhibition & Coffee Break		
	15:10–18:00	15:20–16:30		
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		16:30–19:30		
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18:00–20:00 Welcome Reception p. 14			19:30–02:00 Social Evening & Party p. 21	

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Venue
University of Milan
Via Festa del Perdono 7 • 20122 Milan (Italy)

Date
14–18 June 2015

Conference Website
www.bageco2015.org

Conference Chair
Daniele Daffonchio
University of Milan
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and
King Abdullah University of Science and Technology (KAUST)
BESE • Biological and Environmental Sciences and Engineering Division
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Dear colleagues,

The changing environments hide microbial continuities that are actually dissected following the novel approaches integrating 'metaomics' and novel cultivation based strategies. The 13th meeting on Bacterial Genetics and Ecology (BAGECO) intends to provide a forum for discussing all the aspects related to such microbial continuities along changing environments including large and small-scale gradients, chemoclines and environmental transitions. Biotic gradients, like those in the animal gut, and abiotic ones, where physicochemical variations occur, as well as the combination of the two in relation to any complex ecosystems will be central focuses of the conference.

We are pleased to welcome some of the most renowned scientists in microbial ecology to discuss all these aspects in relation to the most complex environments of earth including, among others, soil, sediments, the symbiotic ecosystems associated to plant, animals and humans, the aquatic ecosystems and the role of microbes and their metabolism in the protection of the environment. The conference debate will be also discussing the advancement on our understanding of the role of gene flow and on how passing from empirical approaches to dissect microbial community functions to establishing the theories that rule out the microbial world and finally the functioning of earth.

We are pleased that you are joining BAGECO 13 to report and learn about these topics and to enjoy the social networking with the scientific microbial ecology community in the wonderful city of Milan.

We are happy to welcoming you to Milan.

Professor Dr. Daniele Daffonchio
Conference Chair

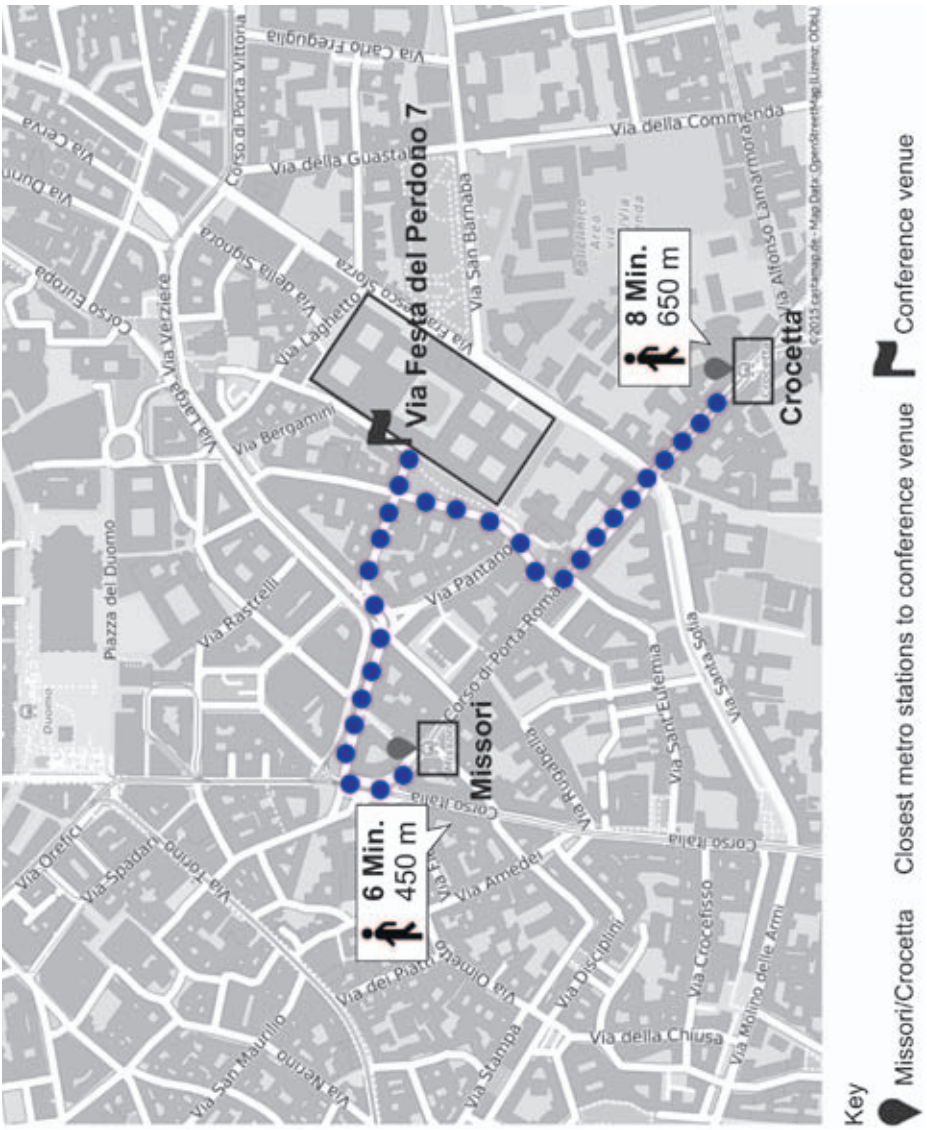
Opening Hours

	Sunday	Monday	Tuesday	Wednesday	Thursday
Industrial Exhibition	–	10:00–18:00	10:30–19:30	10:00–15:30	–
Poster Exhibition	–	15:00–18:00	08:30–19:30	08:30–18:00	–
Check-In	16:00–20:00	08:00–18:00	08:00–19:30	08:00–15:30	08:30–13:30
Media Check-In	16:00–19:00	08:00–18:00	08:00–19:30	08:00–15:30	08:30–11:00

Certificate of Attendance
Certificates of attendance will be available on the last day of the conference at the check-in desk.

Name Badge
Please wear your name badge during all conference events, including the welcome reception and conference dinner. Admission to scientific sessions and to the industrial exhibition is restricted to participants wearing their badge.

Internet Lounge and WIFI Access
The internet lounge is located in the foyer area just outside the plenary hall "Aula Magna". WIFI is available for free within the whole conference area.
Network name: unimi-convegni (no password required)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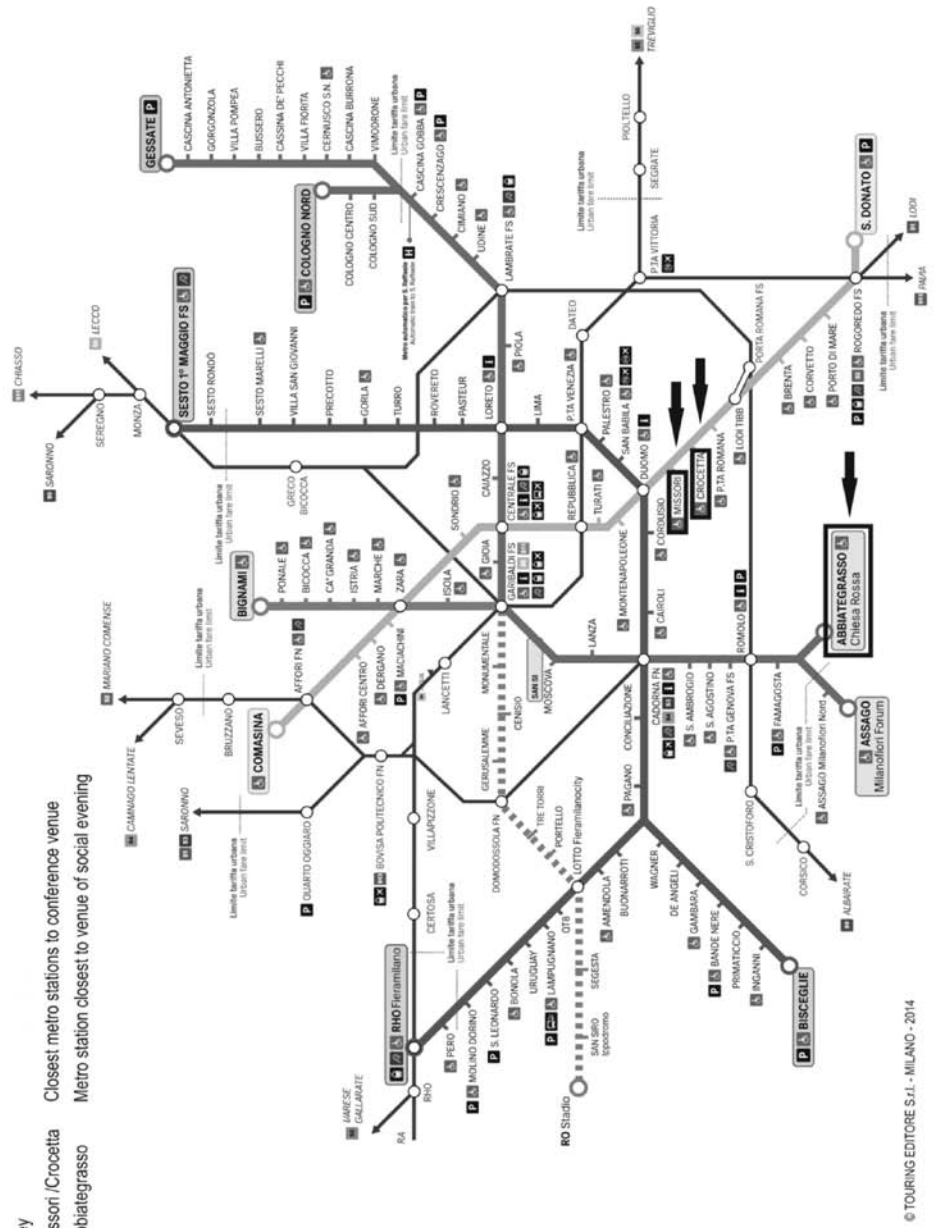
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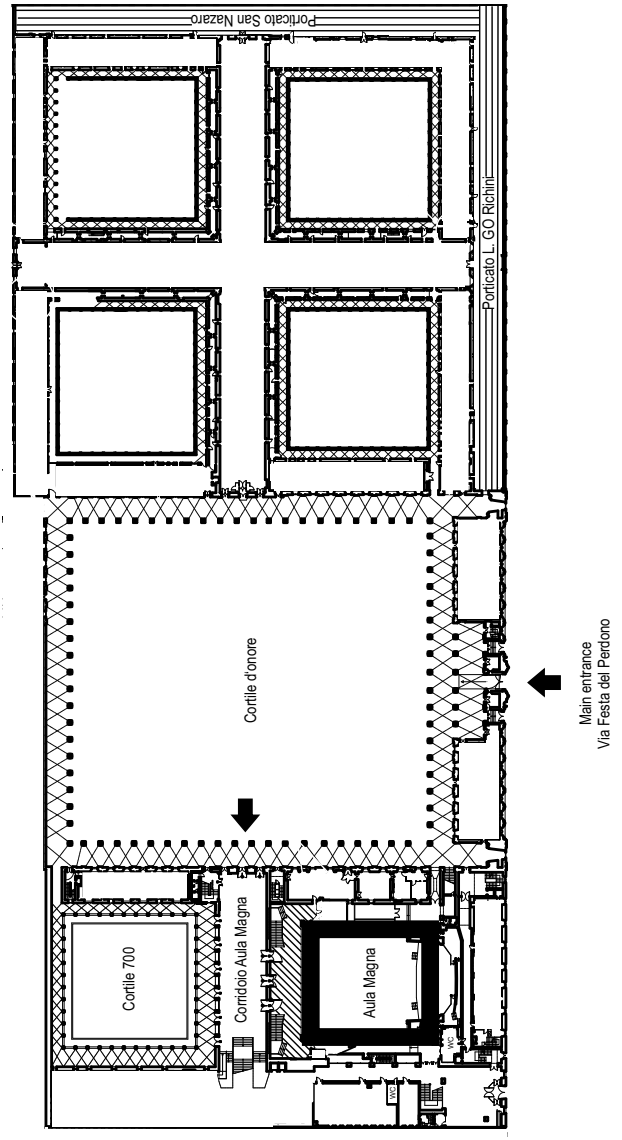

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A presentation notebook with a PDF reader and MS Office PowerPoint 2010/2007 will be provided. The use of personal notebooks is possible upon agreement. However, it may interrupt the flow of the program in the lecture hall. Please provide an adapter for VGA if necessary. To guarantee a smooth running program please upload your presentation on time – at least 2 hours before your presentation starts.

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For submission, please use a USB flash drive, CD or DVD disc that is not protected by any software. Professional staff and equipment will be available for you to arrange and preview your presentation.

Time Allotment
Please prepare your presentation for the allotted amount of time. Chairs and moderators may interrupt should you overrun your time limit. Speaking time is assigned as follows (speaking + discussion time):

Keynote Speakers	25 + 5 minutes
Abstract Authors	15 + 5 minutes

Poster Sessions
Posters should be no larger than DIN A0 (84.1 cm x 118.9 cm). Poster pinboards are 120 cm x 150 cm. Those are only to be used with the designated pins. Pinboards will be numbered. You will find your poster number in the program book on pages 24-52.

All posters are displayed in the hallways "porticato L. GO Richini and San Nazaro". Please see page 9 for exact location.
Poster presenters are asked to be present during their poster session.

Please note that posters should be erected on **15 June by 14:00 hrs** and removed on **17 June by 18:00 hrs**.
All posters that have not been removed by then will be considered as waste.

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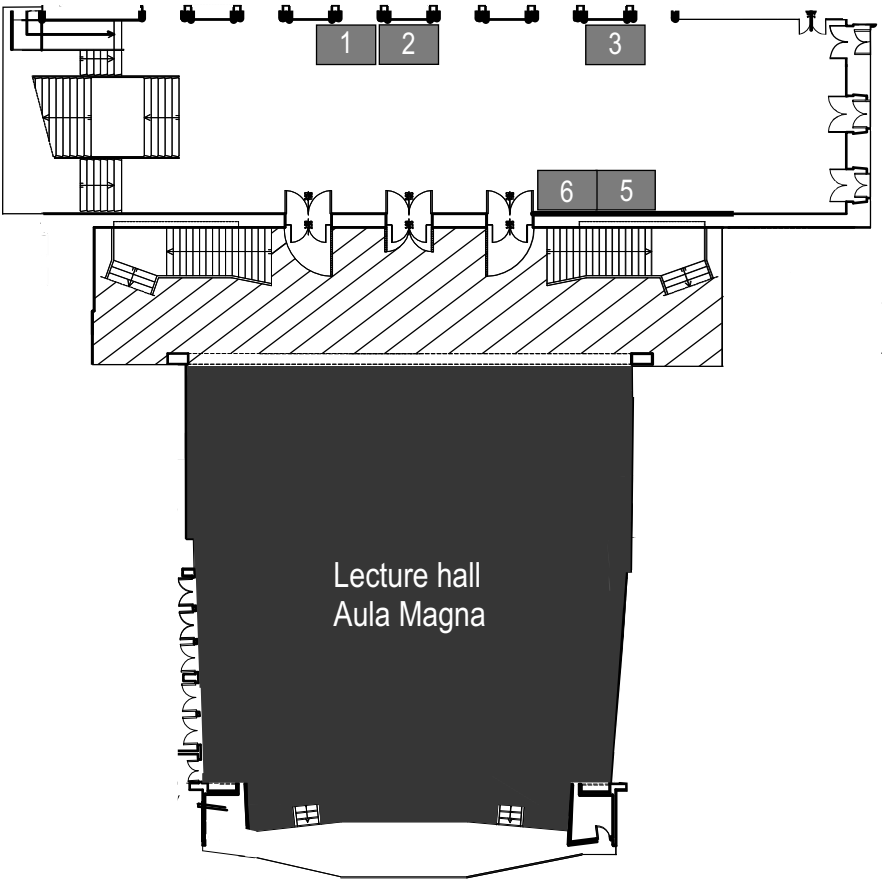

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5 Applied Maths NV
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Welcome Reception

The Welcome Reception of BAGECO 13 will be hosted within the amenities of the University of Milan. All participants are welcome to join their friends and colleagues for the Welcome gathering in the frame of the 13th Symposium on Bacterial Genetics and Ecology.
Some snacks and drinks will be provided.

Date	Sunday, 14 June 2015
Time	18:00–20:00 hrs
Venue	University of Milan • Via Festa del Perdono 7
Price	included for conference participants 20 EUR for accompanying persons

Conference dinner and party

The Maison Milano provides a warm, elegant and comfortable atmosphere for our BAGECO conference dinner and party. Moreover, the in-house kitchen offers a journey through Italian flavors with a rich choice of typical dishes. After dinner and in representative BAGECO tradition you will have the chance to enjoy some great music and to round off the day dancing and chatting with your colleagues.

Date	Wednesday, 17 June 2015
Time	19:30–02:00 hrs
Price	50 EUR
Venue	Maison Milano • Via Montegani 68 • 20141 Milano
Metro	The closest metro station is the final station of line 2 (green line) "Abbiategrasso". From there it is only 350 metres (4 min walk).

17:00–17:30 **Opening of BAGECO 13**
Daniele Daffonchio, Italy

17:30–18:00 **Opening Lecture**
KN 1 • Biological soil crust – ancient and widespread microbial communities
Ferran Garcia-Pichel, United States

18:00–20:00 **Welcome Reception at University of Milan**
see page 13

DID YOU KNOW?

Conventus is the professional congress organiser for the

13th Symposium on Bacterial Genetics and Ecology from 14—18 June 2015 (BAGECO).

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CONGRESSMANAGEMENT

08:30–10:20 **Microbial diversity and functioning in the soil ecosystem**
Chair Jan Dirk van Elsas, The Netherlands

08:30 **Keynote Lecture**
KN 2 • Microbiome assisted protection of plant roots
Gabriele Berg, Austria

09:00 **O SOIL 1 • Plant root exudates shape the diversity of denitrifying bacteria and stimulate their activity**
Feth-el-Zahar Haichar, France

09:20 **O SOIL 2 • The role of *Desulfitobacterium* spp. in the global network of *O*-demethylation in soil**
Felix Mingo, Germany

09:40 **O SOIL 3 • The power of the rare: sulfate reduction in an acidic peatland is driven by small networks of natively low abundant bacteria**
Michael Pester, Germany

10:00 **O SOIL 4 • *Miscanthus x giganteus* crops improve microbial patrimony in polluted soils**
Emilie Bourgeois, France

10:20 Industrial Exhibition & Coffee Break

10:50–12:40 **Microbial diversity and functioning in soil and non-conventional ecosystems**
Chair Pascal Simonet, France

10:50 **Keynote Lecture**
KN 3 • Microbial diversity and function in Arctic ice and snow
Catherine Larose, France

11:20 **O NON 1 • Dissimilatory nitrogen reduction of soil fungal community members and their possible contribution to N2O production in soil**
Christoph Keuschnig, France

11:40 **O NON 2 • Growing up in a tough neighbourhood: adaptations of ammonia oxidising archaea enabling growth at low pH**
Graeme Nicol, France & United Kingdom

12:00 **O NON 3 • Persistence of the dominant soil phylum Acidobacteria by trace gas scavenging**
Sergio Morales, New Zealand

12:20 **O NON 4 • Tropical soil-borne microbiome: linking diversity to function**
Eiko Kuramae, The Netherlands

12:40 Industrial Exhibition & Lunch Break

13:40–15:10 Symbiosis as a driving force of ecosystems I
Chair Gabriele Berg, Austria

13:40 **Keynote Lecture**
KN 4 • Arbuscular mycorrhizal fungi and their endobacteria – a symbiotic microbiota
Paola Bonfante, Italy

14:10 **O SYMl 1 • Functioning of lichen symbioses is supported by a diversified bacterial microbiome**
Martin Grube, Austria

14:30 **O SYMI 2 • Biological activity and colonization pattern of the beneficial endomycotic bacterium *Rhizobium radiobacter* F4 in plant roots**
Stefanie P. Glaeser, Germany

14:50 **O SYMl 3 • Fate of pathogenic *Bacillus cereus* spores after ingestion by protist grazers**
Anne Winding, Denmark

15:10–18:00 Poster Session I

Topics
Microbial diversity and functioning in the soil ecosystem see page 24
Symbiosis as a driving force of ecosystems see page 34

08:30–10:40 **Symbiosis as a driving force of ecosystems II**
Chair Christoph C. Tebbe, Germany

08:30 **Keynote Lecture**
KN 5 • Gendercide symbionts and hopeful symbiotic monsters, in natural and man-made ecosystems
Claudio Bandi, Italy

09:00 **O SYMII 1 • Meeting the aliens: morphogenesis induction of the green alga *Ulva mutabilis* by sponge-associated bacteria highlights functional redundancy as a key factor shaping marine holobiont communities**
Rodrigo Costa, Portugal

09:20 **O SYMII 2 • Nitrogen-fixing symbioses between unicellular organisms are critical for open ocean ecosystems**
Jonathan Zehr, United States

09:40 **O SYMll 3 • Nutritional complementation in an insect-bacterial symbiosis – un ménage à trois**
Bessem Chouaia, United States

10:00 **O SYMII 4 • *Spiroplasma*, a new symbiont in tsetse flies**
George Tsiamis, Greece

10:20 **O SYMII 5 • Honeybee symbionts protect their host from American Foulbrood disease**
Elena Crotti, Italy

10:40 Industrial Exhibition & Coffee Break

11:10–12:20 **The food to gut microbial continuity I**
Chair Marco Bazzicalupo, Italy

11:10 **Keynote Lecture**
KN 6 • How gut microbiota impacts host growth and immunity: Lesson from a Drosophila model
Won-Jae Lee, South Korea

11:40 **O FOO 1 • How mutualism evolves: experimental microbiome evolution in gnotobiotic flies**
Maria Elena Martino, France

12:00 **O FOO 2 • A mechanistic view of methanogenic modulation in the rumen**
Nir Friedman, Israel

12:20 Industrial Exhibition & Lunch Break

13:20–14:50 **The food to gut microbial continuity II**
Chair Janet Jansson, United States

13:20 **Keynote Lecture**
KN 7 • The food-human axis: dietary habits and gut microbiota and Metabolome
Marco Gobbetti, Italy

13:50 **O FOOD 1 • Indonesian Tempeh as functional food: role of bacterial community as revealed by conventional microbiology and next generation sequencing analysis**
Antonius Suwanto, Indonesia

14:10 **O FOOD 2 • Comparative metagenomics from oral microbiomes of hunter-gatherer and farmer populations from the Philippines**
Florent Lassalle, United Kingdom

14:30 **O FOOD 3 • Gut microbiota diversity of omnivore, vegetarian and vegan healthy subjects by culture dependent and rRNA DGGE profiling**
Ilario Ferrocino, Italy

14:50 Industrial Exhibition & Coffee Break

15:20–16:30 **Round Table: from microbial communities to single cells and back**
Moderator Janet Jansson, United States

15:20 **KN 8 • Multi-omics of the human gut microbiome**
Janet Jansson, United States

15:30 **KN 9 • Soil metagenomics and forensics**
Pascal Simonet, France

15:40 **KN 10 • Why Metagenomics?**
Timothy M. Vogel, France

15:50 **KN 11 • Massive parallel amplicon sequencing as a means to reveal niche preferences and eco-physiological properties of uncultured microbial taxa**
Christoph C. Tebbe, Germany

16:00 **Discussion**

16:30–19:30 Poster Session II

Topics

08:30–10:20 **Gradients in conventional and extreme habitats**
Chair Daniele Daffonchio, Italy

08:30 **Keynote Lecture**
KN 12 • The microscale dynamics of ocean microbes
Roman Stocker, United States

09:00 **O GCEH 1 • Dissecting massive methanotrophic biofilm formation in a mineral spring cavern**
Clemens Karwautz, Germany

09:20 **O GCEH 2 • Effects of alternative water events on soil microbial communities from contrasting aridity zones of the Namib Desert**
Aline Frossard, South Africa

09:40 **O GCEH 3 • Growth of Geobacter metallireducens under environmental conditions with carbon limitation in sediment columns indicates new types of regulation of catabolic pathways**
Sviatlana Marozava, Germany

10:00 **O GCEH 4 • The effect of adhesion forces between bacteria and anode on electron transfer in microbial fuel cells**
Alexiane Godain, France

10:20 Industrial Exhibition & Coffee Break

10:50–12:20 **Novel biodegradation pathways along spatial gradients I**
Chair Amalia Karagouni, Greece

10:50 **Keynote Lecture**
KN 13 • "Omics" and microbial ecology – opening new windows of research
Manuel Ferrer Martinez, Spain

11:20 **O NOVl 1 • Biodegradation of naphthenic acids by *Rhodoccocus spp*.**
Stefano Fedi, Italy

11:40 **O NOVl 2 • Co-oxidation of dimethylsulfide to dimethylsulfoxide by marine heterotrophic bacteria using trimethylamine monooxygenase**
Ian Lidbury, United Kingdom

12:00 **O NOVl 3 • Biogeophysics as a tool for "Smart Sampling" during the microbial degradation of hydrocarbons**
Silvia Rossbach, United States

12:20 Industrial Exhibition & Lunch Break

13:20–15:10 Adaption and the role of HGT
Chair Elizabeth Wellington, United Kingdom

13:20 **Keynote Lecture**
KN 14 • Diverse contributions of IncP-1 plasmids to bacterial adaptation in the environment
Kornelia Smalla, Germany

13:50 **O HGT 1 • Selection for horizontal gene transfer shapes the social game between honest and eavesdroppers in constructed communities of *Bacillus subtilis***
Ines Mandic-Mulec, Slovenia

14:10 **O HGT 2 • Bistable Integrative and conjugative element transfer in *Pseudomonas* is controlled by local nutrient levels**
François Delavat, Switzerland

14:30 **O HGT 3 • c-di-GMP related genes are common on plasmids – a comparative analysis**
Samuel Jacquiod, Denmark

14:50 **O HGT 4 • Metal stress modulates the immediate plasmid uptake potential of soil microbes**
Uli Klümper, Denmark

from 15:10 Free afternoon

from 19:30 Social Evening & Party at Maison Milano
see page 13

09:00–10:30 **Novel biodegradation pathways along spatial gradients II**
Chair Timothy M. Vogel, France

09:00 **Keynote Lecture**
KN 15 • Ecological concepts for anaerobic degradation of aromatic hydrocarbons in groundwater and oil reservoirs
Rainer Meckenstock, Germany

09:30 **O NOVII 1 • Characterization of anaerobic BTEX-degrading enrichment cultures from groundwater along a pollutant gradient**
Martin Sperfeld, Germany

09:50 **O NOVII 2 • Unusual bacterial community assembly of a simplified consortium reductively dechlorinating 1,2-dichloroethane**
Giuseppe Merlino, Saudi Arabia

10:10 **O NOVII 3 • Metagenomic and metagenic approaches applied to enhanced anaerobic reductive dechlorination of polychlorinated biphenyls: linking structure and function**
Sebastien Cecillon, France

10:30 Industrial Exhibition & Coffee Break

11:00–12:30 **Constructed microbial communities as a tool in microbial ecology**
Chair James Prosser, United Kingdom

11:00 **Keynote Lecture**
KN 16 • Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities
Nico Boon, Belgium

11:30 **O CMC 1 • Noise and competition: bacteriocin role in *Escherichia coli* populations**
Bihter Bayramoglu, Israel

11:50 **O CMC 2 • Testing neutral and niche processes which shape microbial communities**
Antonis Chatzinotas, Germany

12:10 **O CMC 3 • High-wire acts: Mycelia as hot spots for horizontal gene transfer of bacteria**
Tom Berthold, Germany

12:30 **Closing Lecture**
Getting to know our sustaining microbiome
James Tiedje, United States

13:00 **Farewell and announcements**
Daniele Daffonchio, Italy

15:10–18:00 **Poster Session I**

Topics

Microbial diversity and functioning in the soil ecosystem

P SOIL 1 The impact of forest logging and oil palm plantations on microbial diversity and activity in an Indonesian tropical peat swamp soil
Yuana Nurulita (Bundoora Pekanbaru – Riau Bundoora/Australia)

P SOIL 2 Proteomics reveals the impacts of deforestation on bacterial diversity and carbon fixation processes under arid climate
Felipe Bastida (Murcia Albacete/Spain)

P SOIL 3 Beyond the extracellular-ecosystem: linking community variations, cellular functionality and phyla lifestyles of restored drylands
Felipe Bastida (Murcia Albacete/Spain)

P SOIL 4 The cyanobacterial diversities of two different geothermal hot springs in Thailand
Nuannoi Chudapongse (Nakhon Ratchasima/Thailand)

P SOIL 5 Purification and identification of an antimicrobial and antitumoral agent isolated from *B. safensis*
Tiago Silva (Campinas/Brazil)

P SOIL 6 Phylogenetic analysis of antimicrobial-producing Actinomycetes isolated from dry dipterocarp forest soil in northeast Thailand
Nawarat Nantapong (Nakhon Ratchasima/Thailand)

P SOIL 7 Zinc-lead mine soils shape genetic diversity and distribution of *Anthyllis vulneraria* rhizobial symbionts
Roba Mohamad (Montpellier/France)

P SOIL 8 Towards net zero/negative methane emission in agricultural soils: Unexpected atmospheric methane consumption after amendments with specific residues
Adrian Ho (Wageningen/The Netherlands)

P SOIL 9 Microbial community structure doesn't matter in litter decomposition
Caio Rachid (Rio de Janeiro/Brazil)

ProkaGENOMICS 2015

6th European Conference on Prokaryotic and Fungal Genomics

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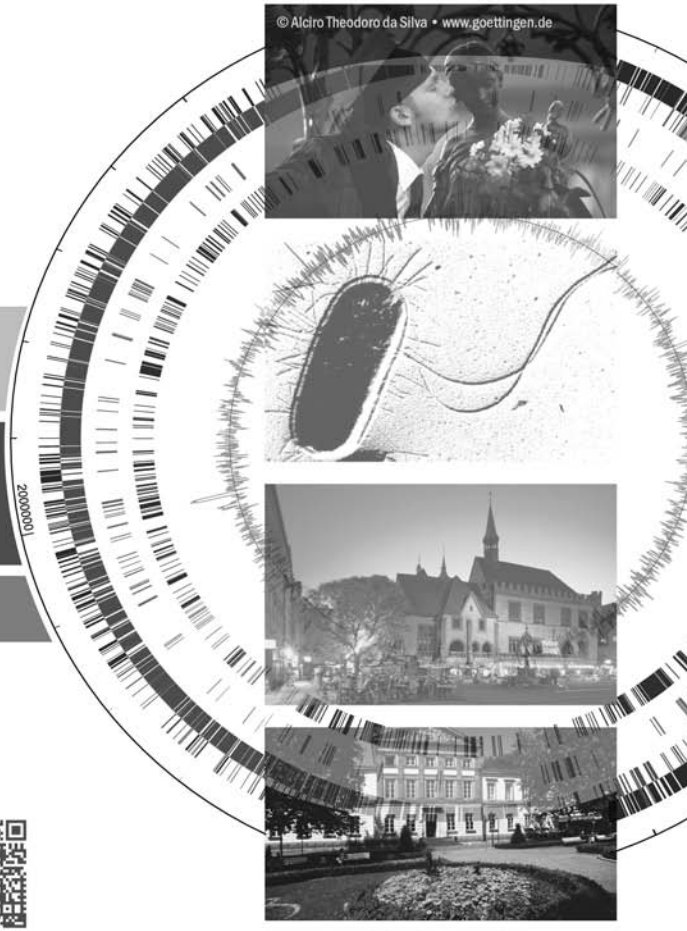

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P SOIL 10 Removal of Geosmin by a bacterium isolated from biological activated carbon
Jaewon Lim (Wonju-si, Gangwon-do/Republic of Korea)

P SOIL 11 Analysis of attached bacterial communities during biological activated carbon process in drinking water treatment plants
Jaewon Lim (Wonju-si, Gangwon-do/Republic of Korea)

P SOIL 12 Bacterial community responses in mixed forestry plantation of *Eucalyptus* and *Acacia*
Helena DM Villela (Rio de Janeiro/Brazil)

P SOIL 13 Assessing the composition of bacterial community associated with different types of deadwood
Vojtech Tlaskal (Prague/Czech Republic)

P SOIL 14 Multi-analytical approach reveals potential microbial indicators in soil for sust ainable sugarcane model systems
Acacio Aparecido Navarrete (Piracicaba/Brazil)

P SOIL 15 Effects of biochar addition on bacterial populations of the lettuce and strawberry rhizosphere
Caroline De Tender (Merelbeke Ghent/Belgium)

P SOIL 16 Plant bacterial inoculants as invaders on crop fields: Invasion ecology applied to rhizosphere microbiomes
Pedro Beschoren da Costa (Porto Alegre/Brazil, Bielefeld/Germany)

P SOIL 18 A functional view of mangrove microbiomes trhough meta-omics
Armando Cavalcante Franco Dias (Piraicicaba/Brazil)

P SOIL 19 Isolation and characterization of an endophytic bacterium, Bacillus megaterium BMN1, associated with root-nodules of Medicago sativa L. growing in Al-Ahsaa region, Saudi Arabia
Ashraf Khalifa (Al Ahsaa Beni-Suef/Saudi Arabia)

P SOIL 20 Bacterial and archaeal diversities in San Kamphaeng hot spring, Chiangmai, Thailand
Oratai Weeranantanapan (Nakorn Ratchasima/Thailand)

P SOIL 21 Effect of different organic amendments on soil microbial communities
Sophie Sadet-Bourgeteau (Dijon/France)

P SOIL 22 Changes in Cerrado biome microbial communities' composition and function in response to seasonal variations in water availability.
Ricardo Kruger (Brasilia/Brazil)

P SOIL 23 Temperature effects on recovery time of bacterial growth after rewetting of dry soil
Anita Maienza (Florence/Italy)

P SOIL 24 Bacterial enzyme systems for the decomposition of cellulose and Hemicelluloses contained in plant biomass from forest soil are complex and highly diverse
Ruben Lopez-Mondejar (Prague/Czech Republic)

P SOIL 25 Accessing the microbiome of Brazilian sugarcane soils through metagenomics
Júlia Lima-Perim (Piracicaba/Brazil)

P SOIL 26 Concentration-modulated growth in reduced communities: Distinguishing responses of soil microorganisms by micro segmented flow
Johann Michael Koehler (Ilmenau/Germany)

P SOIL 27 Comparative evaluation of bacterial diversity from GM and Non- GM Maize rhizosphere
Naseer Ahmad (Abbottabad/Pakistan)

P SOIL 28 Control of boreal forest soil decomposition processes by plant secondary compounds
Mary-Cathrine Leewis (Fairbanks/United States)

P SOIL 29 Diversity and functional analysis in Brazilian biomes
Luciano Takeshi Kishi (Jaboticabal/Brazil)

P SOIL 30 Maize lines with different Nitrogen use efficiency (NUE) differ the molecular diversity of β-glucosidase encoding genes and glycosidase activity in their rhizosphere
Shamina Pathan (Florence/Italy)

P SOIL 31 A degradation pathway for sulfoquinovose in a typical soil bacterium, *Pseudomonas putida* SQ1, via a so-called Entner-Doudoroff type pathway
Ann-Katrin Felux (Konstanz/Germany)

P SOIL 32 Fungal-bacterial interplays at biogeochemical interfaces:Co-occurrence of fungi and bacteria in natural and artificial soils
Annelie Steinbach (Leipzig/Germany)

P SOIL 33 Gene capture by hybridization as a strategy for targeted microbial genome reconstruction
Cyrielle Gasc (Clermont Ferrand/France)

P SOIL 34 Unrevealing the ecological secrets of important bacterial taxa from forest soil through isolation, whole genome sequencing and single-cell genomics
Salvador Lladó (Prague/Czech Republic)

P SOIL 35 Rhizosphere microbial community composition of common beans with different levels of resistance to Fusarium oxysporum
Lucas William Mendes (Piracicaba/Brazil)

P SOIL 36 Microbial diversity in Brazilians soils
Camila Fernandes (Jaboticabal/Brazil)

P SOIL 37 Unravelling the functional bacterial diversity from different soils supplemented with organic fertilizer from a Brazilian zoo park
Lucia Maria Carareto Alves (Jaboticabal/Brazil)

P SOIL 38 Biodegradative abilities of novel fungus isolated Aspergillus sp. SIL2014 from environmental habitats soil contaminated automotive lubricants able to use a lot of hydrocarbons as only carbon and energy sources
Eliana Gertrudes de Macedo Lemos (Jaboticabal/Brazil)

P SOIL 39 Characterisation of Escherichia coli B2 strains from waters of Sydney and Gold Coast regions
Angelin Samuel (Canberra/Australia)

P SOIL 40 The diversity and abundance of phytase genes (BPP) in the maize rhizosphere
Simone Raposo Cotta (Piracicaba/Brazil)

P SOIL 41 Edaphic and plant associated factors drive diazotroph diversity and function in Australian soils
Vadakattu Gupta (Urrbrae/Australia)

P SOIL 42 Metatranscriptomic sequencing reveals differential expression response of paddy soil microorganisms to salt stress
Jingjing Peng (Marburg/Germany)

P SOIL 43 Diversity of bacteria associated with natural vegetation of long-term PCB-contaminated soil
Lucie Musilova (Prague/Czech Republic)

P SOIL 44 Study of bacterial communities in soils of the Sierra Nevada National Park and rhizosphere of a wild thyme species along a thermoclimatic gradient
Pieter van Dillewijn (Granada/Spain)

P SOIL 45 Characterization of a putative novel *Rhodobacter* species isolated from polluted sediment
Jachym Suman (Prague/Czech Republic)

P SOIL 46 The role of microbial community in degrading leaves in forest environments
Alessia Bani (Bolzano/Italy)

P SOIL 48 The presence of PKS Polyketide Synthase (PKS) and Non-ribosomal Peptide Synthase (NRPS) genes at a deep-sea hydrothermal field in the Aegean Sea
Maria Bourbouli (Athens/Greece)

P SOIL 49 Greek streptomycetes are recruited to fight as biocontrol agents against phytopathogenic fungi
Grammatiki Kanini (Athens/Greece)

P SOIL 50 Effect of biofumigant treatment with defatted seed meals on soil microbial communities
Carolina Chiellini (Florence/Italy)

P SOIL 51 Tapping bacterial resources – Accessing secondary metabolites of the uncultivated
Maja Plesko (Tulln/Austria)

P SOIL 52 Elucidating the role of Microbacterium spp. in the mobilization/immobilization of heavy metals
Erika Corretto (Tulln/Austria)

P SOIL 53 Effects of pasture restoration on soil bacterial community in the Cerrados of Central Brazil
Maria Regina Sartori (Brasilia/Brazil)

P SOIL 54 Search for highly copper-Tolerant soil bacteria by cultivation in micro segmented flow
Johann Michael Koehler (Ilmenau/Germany)

P SOIL 55 Role of soil microbial communities on suppression of plant pathogenic nematodes
Leo van Overbeek (Wageningen/The Netherlands)

P SOIL 56 An effective approach to retrieve culturable *Acinetobacter* spp. from the soil environment
Lenka Krizova (Prague/Czech Republic)

P SOIL 57 Seasonal dynamics of soil microbial communities under dominant understory vegetation in spruce swamp forest
Alica Chronakova (Ceske Budejovice/Czech Republic)

P SOIL 58 Comparison of bacterial soil communities between vineyards and their surrounding semi-natural areas.
Marina Zanardo (Legnaro/Italy)

P SOIL 59 Soil archaeal community changes associated with oil palm fatal yellowing using pyrotags
Ricardo Kruger (Brasilia/Brazil)

P SOIL 60 Soil organic matter mineralization depends on microbial diversity
Pierre-Alain Maron (Dijon/France)

P SOIL 61 Non-conventional pretreatments mitigate the inhibitory effect of 5-hydroxymethylfurfural in dark fermentation process
Micol Bellucci (Foggia/Italy)

P SOIL 62 Emissions of Volatile Organic Compounds (VOCs) from soil to the atmosphere depending on agricultural land-uses – Interrelationships between SOM, microbial diversity and VOCs fluxes
Kevin Potard (Rennes/France)

P SOIL 63 Diversity analysis and mining of functional genes from soil metagenomes – case study with ring hydroxylating oxygenases
Ondrej Uhlik (Prague/Czech Republic)

P SOIL 64 Rhizosphere microbiome as affected by plant species, Fe nutrition and growth ubstrates
Youry Pii (Bolzano/Italy)

P SOIL 65 The effect of plant nutritional strategy on the investment into exudation, and the consequences on denitrifying community
Julien Guyonnet (Villeurbanne/France)

P SOIL 66 Culturable endophytic bacteria from salt marsh plant *Halimione portulacoides*: functional characterization, phylogenetic diversity and influence of metal contamination
Cátia Fidalgo (Aveiro/Portugal)

P SOIL 67 Diversity of novel alkaline protease producing bacteria from Chilika Lake, Odisha, India
Ananta Narayan Panda (Bhubaneswar, Odisha/India)

P SOIL 68 Metagenomic analysis of microbes living in Mars analogue environments
Alexandra Perras (Graz/Austria, Regensburg/Germany)

P SOIL 69 Functional and structural characterization of lipolytic enzymes soil metagenomics contaminated with waste lubricant to obtain proteins with high biotechnological potential
Aliandra Maura Gibertoni (Jaboticabal/Brazil)

P SOIL 70 Networks and co-occurrence relationships in the lettuce root microbiota
Martin Grube (Graz/Austria)

P SOIL 71 Genome sequencing of Microbacteriaceae spp. with emphasis on heavy metal contaminated environments.
Livio Antonielli (Tulln/Austria)

P SOIL 72 Hitherto the analysis of genome fragments from bacteria involved in sulphur cycling in mangrove soils Marcus Venicius
Mello Lourenco (Piracicaba/Brazil)

P SOIL 73 Plants as drivers of bacterial community structure in aged contaminated soil
Ondrej Uhlik (Prague/Czech Republic)

P SOIL 74 High taxonomic diversity of culturable Acinetobacter bacteria in the natural soil environment
Alexandr Nemec (Prague/Czech Republic)

P SOIL 75 Metatranscriptome based analysis of hydrolytic microbial communities in an acidic Northern peatland
Anastasia Ivanova (Moscow/Russian Federation)

P SOIL 76 Effect of phenanthrene on mobile organic matter and bacterial communities in soil
Doreen Babin (Braunschweig, Jena/Germany)

P SOIL 77 Expression and purification of novel chitinases from metagenomics
Francesca Berini (Varese/Italy)

P SOIL 78 Expression and purification of a metagenome-sourced laccase
Francesca Berini (Varese/Italy)

P SOIL 79 Factors influencing the fate of human pathogens in the plant environment
Eva Fornefeld (Braunschweig/Germany)

P SOIL 80 Novel insights into the *Eruca sativa* microbiome and the function of *Enterobacteriaceae*
Armin Erlacher (Graz/Austria)

P SOIL 81 Screening for Ligninolytic Enzymes in metagenomic Libraries from Caatinga semi-arid soil by function and sequenced based approaches
Gileno Vieira Lacerda Júnior (Paulínia/Brazil)

P SOIL 82 Soil and plant associated bacterial communities in two different successional stages of sub-Arctic sand dune ecosystem
Anbu Poosakkannu (Jyväskylä/Finland)

P SOIL 83 Evaluation of the influence of different corn-based cropping systems on the biodiversity of the soil bacterial communities
Zita Sasvári (Gödöllő/Hungary)

P SOIL 84 Effect of nitrogen ultilizing effeciencies on rhizospheric proteolytic bacterial communities of two maize inbred lines
Divyashri Baraniya (Florence/Italy)

P SOIL 85 Hunting down frame shifts – Processing of functional gene amplicon sequences
Michal Strejček (Prague/Czech Republic)

P SOIL 86 Microbial diversity of different sugarcane vinasse
Matheus Cipriano (Wageningen/The Netherlands)

P SOIL 87 Extreme concentration of biochar: dynamics of microbial response in pre-exposed and pristine soils
Valentina Imparato (Roskilde/Denmark)

P SOIL 88 Influence of different phosphate sources on the bacterial microbiome in the rhizosphere and endorhiza of barley (*Hordeum vulgare* L.), investigated by rRNA-deep sequencing
Massimiliano Cardinale (Giessen/Germany)

P SOIL 89 Genomic characterization of a divergent genotype of citrus endophytic *Curtobacterium* strain ER1.6/6
Leandro Maza Garrido (São Paulo/Brazil)

P SOIL 90 Microbial dynamics in the soil-rhizosphere interface: from patterns to ecological processes
Dennis Goss de Souza (Piracicaba/Brazil, Davis/ United States)

P SOIL 91 Exploring ComQXPA quorum sensing diversity and biocontrol potential of *Bacillus* spp. isolates from tomato rhizoplane
Ines Mandic Mulec (Ljubljana/Slovenia)

P SOIL 92 Phylogenetic identification of mungbean-nodulating strain MN-S and characterization of its Nod factors
Mohsin Tariq (Berkeley/United States, Faisalabad/Pakistan)

P SOIL 93 Continuous flooding selects for bacterial populations involved in arsenic cycle in rice rhizosphere
Sarah Zecchin (Milan/Italy)

P SOIL 94 Screening for hydrocarbon-producing bacteria from Antartic marine samples
Michel Passarini (Foz do Iguaçu/Brazil)

P SOIL 95 Characterization of Plant Growth Promoting Bacteria activities
Carmine Crecchio (Bolzano/Italy)

P SOIL 96 Ecology of aerobic and anaerobic ammonia oxidizers communities driven by different soil management in a temperate paddy field
Maria Alexandra Cucu (Turin/Italy)

P SOIL 97 Bacterial assemblages associated with unique root morphology of a desert plant
Ramona Marasco (Thuwal/Saudi Arabia)

P SOIL 98 Bacteria, mineral soil and pioneer plants: a complex interaction in successional stages on Alps
Lorenzo Brusetti (Bolzano/Italy)

P SOIL 99 A pan genome analysis of fluorescent Pseudomonads in sugarcane soil and rhizosphere
Michele C Pereira e Silva (Piracicaba/Brazil)

P SOIL 100 Archaeal and bacterial metabolically active cells depending on soil depth and land use
Mikhail Semenov (Moscow/Russian Federation)

P SOIL 101 Sugarcane harvest management alters soil bacterial communities
Fabiana de Souza Cannavan (Piracicaba/Brazil)

P SOIL 102 Temporal study of phytoremediation revealed a secondary succession of bacteria and novel extradiol dioxygenase genes in hydrocarbon pollution
Kim Yrjälä (Helsinki/Finland)

P SOIL 103 Multiple semi-continuous chemical detection by bacterial bioreporters in a microfluidics chemostat
Siham Beggah Möller (Lausanne/Switzerland)

P SOIL 104 Characterization of test of actinomyctes from different culture systems from Ouargla (Algeria)
Aloui Nabiha (Ouargla/Algeria)

P SOIL 105 The dynamics of physical phenomenon and chemicals in the high plateaus: the case of the province of Algeria Tissemsilt (soil, water, erosion, pollution)
Ouabel Habib (Tiaret/Algeria)

P SOIL 106 Microbial communities from a transect of non-contaminated to highly hydrocarbon-contaminated soils in King George Island, Maritime Antarctic
Diogo Jurelevicius (Rio de Janeiro/Brazil)

P SOIL 107 Microbial taxonomic structure of different soil types of Russia
Ekaterina Ivanova (Saint-Petersburg/Russian Federation)

Symbiosis as a driving force of ecosystems

P SYM 1 Enrichment experiment reduces diversity and changes microbial interactions in an ultra-oligotrophic environment
Gabriel Yaxal Ponce-Soto (Mexico City/Mexico)

P SYM 2 SYBR Green I based quantative real-time PCR assay for removal rate of adenovirus as surrogate of norovirus using polyolefin microfilter membrane
Jaewon Lim (Wonju-si, Gangwon-do/Republic of Korea)

P SYM 3 New unexpected functions for ACC deaminase genes in the *Sinorhizobium meliloti*
Alice Checcucci (Sesto Fiorentino/Italy)

P SYM 4 The utilization of a degrading and a probiotic consortium of bacteria to improve the health of corals following an oil spill
Henrique Santos (Rio de Janeiro/Brazil)

P SYM 5 Sugar cane bagasse affects bacterial community dynamics in the sheep rumen
Rodrigo Mendes (Jaguariuna/Brazil)

P SYM 7 Do different coral species represent different biotechnological sources?
Caren Vilela (Rio de Janeiro/Brazil)

P SYM 8 Insights into termite symbioses from symbiont genomes and metabolomics
Nathan Lo (Sydney/Australia)

P SYM 9 Entophytic bacteria isolated from the red alga *Laurencia glandulifera* as potential producers of bioactive compounds
George Kapetanakis (Athens/Greece)

P SYM 10 Epi- and endophytic microbial communities of arctic and subarctic peatland mosses
Matthias Winkel (Potsdam/Germany)

P SYM 11 Effect of the plant flavonoid luteolin on *Ensifer meliloti*
Giulia Spini (Florence/Italy)

P SYM 12 Bacterial symbionts associated with a phloem-feeding heteropteran
Yao Xu (Gainesville/United States)

P SYM 13 Functional roles of bacteria in lichen-associated bacteria studied by comparative omics
Martin Grube (Graz/Austria)

P SYM 14 Use of an oligrotrophic culture medium enables captivation of diverse alphaprobacterial lineages from the marine sponge *Spongia* sp
Elham Karimi (Faro/Portugal)

P SYM 15 The importance of biodiversity for the functional performance of microbial communities
Deborah Patsch (Duebendorf/Switzerland)

P SYM 16 The ambivalent interaction between *Stenotrophomonas rhizophila* P69 And *Trichoderma* spp.
Gabriele Alfano (Graz/Austria)

P SYM 17 Hopanoids genes expression during *Methylobacterium mesophilicum* SR1.6/6 citrus interaction
Manuella Nóbrega Dourado-Ribeiro (São Paulo/Brazil)

P SYM 18 The efficiency of sugarcane colonization by arbuscular mycorrhizal fungi is modulated by distinct levels of soil microbial diversity
Dorotéia Alves Ferreira (Piracicaba/Brazil)

P SYM 19 Exploring the hologenome concept in *Venerupis philippinarum* (Manila clam)
Laura Leite (Aveiro/Portugal)

P SYM 20 Bacterial cargo on symbiotic propagules of the lung lichen *Lobaria pulmonaria*
Ines Aline Aschenbrenner (Graz/Austria)

P SYM 21 Diversity of methanogens and co-occurrence with bacteria in feces in humans of different age and health status
Clarissa Schwab (Zurich/Switzerland)

P SYM 22 Molecular "bridges" that build rumen microbial alliances
Dragana Gagic (Palmerston North/New Zealand)

P SYM 23 Illumina sequencing unveils host-specific profiles and highly diversified microbial dark matter in marine sponge symbiont communities
Rodrigo Costa (Faro/Portugal)

P SYM 24 The importance of intraspecific diversity of ectomycorrhizal fungi for regulating ecosystem functioning
Christina Hazard (Ecully Cedex/France, Aberdeen/United Kingdom)

P SYM 25 Comparative genomics reveals shared and specific *Vibrio* strains across multiple animal hosts
Rodrigo Costa (Faro/Portugal)

P SYM 26 Depicting the bacterial community associated with culturable cyanobacteria
Pedro Avelino Maia Andrade (Piracicaba/Brazil)

P SYM 27 Transcriptomic analysis of differential gene expression of Methylobacterium *mesophilicum* SR1.6/6 during citrus interaction
Aline Aparecida Camargo-Neves (São Paulo/Brazil)

P SYM 28 Characterization of the bacterial community associated to Pine Wilt Disease through culture-independent methods
Marta Alves (Aveiro/Portugal)

P SYM 29 Metatranscriptomics on termite and host associated symbionts to identification of biomass-degrading enzymes
Melline Noronha (Paulínia/Brazil)

P SYM 30 Improving truffles development. Rhizosphere and Mycorrhizosphere host different bacterial and fungal consortia.
Anna Sandionigi (Milan/Italy)

P SYM 31 First report of the occurrence of White Pox disease in a scleractin coral *Siderastrea stellata*
Deborah C. A. Leite (Rio de Janeiro/Brazil)

P SYM 32 Alfalfa root symbionts under soil nutrient pressure: cooperation or competition?
Guillaume Lentendu (Halle/Germany)

P SYM 33 Random mutagenesis of endophytic Burkholderia seminalis suggest that the gene cluster related to capsule synthesis is associated to inhibition of plant pathogens
Welington Araujo (São Paulo/Brazil)

P SYM 34 Molecular and ecological interactions between plant pathogen and biocontrol strains of Burkholderia
Emy Tiyo Mano (São Paulo/Brazil)

P SYM 35 Functionality of honeybee (*Apis mellifera*) LAB member symbionts
Irakli Janashia (Tbilisi/Georgia)

P SYM 37 Contribution of gut symbionts to the host physiology of a wood-boring beetle
Erica Prosdocimi (Milan/Italy)

P SYM 38 Bacterial symbiosis in dual-breathing animals living in mangrove ecosystem
Marco Fusi (Thuwal/Saudi Arabia)

P SYM 39 Habitat visualization and genomic analysis of "*Candidatus* Pantoea carbekii", the crypt-dwelling primary symbiont of the brown marmorated stink bug
Zakee Sabree (Columbus/United States)

16:30–19:30 **Poster Session II**

Adaptation and the role of HGT

P HGT 1 Mutagenic processes in environmental strains of pseudomonads
Tanel Ilmjärv (Tartu/Estonia)

P HGT 2 Plasmid pP32BP2 of *Psychrobacter* SP. DAB_AL 32B and Its Role in Host Adaptation to Extreme Arctic Environment
Anna Ciok (Warsaw/Poland)

P HGT 3 Ecology and diversity of Antarctic psychrophilic bacteria isolates from ornitogenic soil
Anna Ciok (Warsaw/Poland)

P HGT 4 Organic fertilizers as a source of resistance genes and mobile genetic elements and their potential contribution to the spread of resistance genes in field soils
Birgit Wolters (Braunschweig/Germany)

P HGT 5 Soil type-dependent effects of streptomycin and doxycycline applied with manure on the structure and resistome of soil bacterial communities
Sven Jechalke (Braunschweig/Germany)

P HGT 6 Analysis of Horizontal Gene Transfer Systems in Members of *Roseobacter* Lineage
Maria Mas-Lladó (Palma/Spain)

P HGT 7 Dissemination of ESBL genes within microbial communities in river sediments
Gemma Hill (Coventry/United Kingdom)

P HGT 8 Transposases and Insertion Sequences in *Ferroplasma acidarmanus* fer 1 shared homology with fifteen *Bacteria and Archaea*
Ravindra Naraine (Grand Anse/Grenada)

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IOBC-WPRS

XIV Meeting of the Working Group Biological control of fungal and bacterial plant pathogens

Biocontrol and Microbial Ecology

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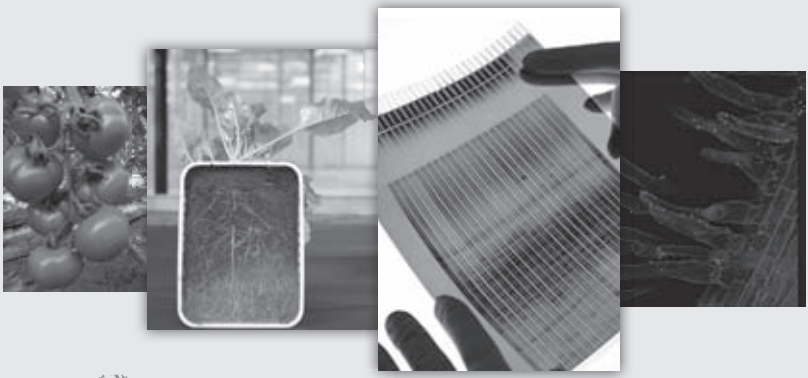

Conference Chairs
Rita Grosch
Leibniz Institute of Vegetable and Ornamental Crops (IGZ)

Kornelia Smalla
Julius Kühn-Institut (JKI)

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P HGT 9 Genome Sequences of Thermophilic Sulfur Disproportionating Bacterium Thermosulfurimonsas Dismutans
Andrei Mardanov (Moscow/Russian Federation)

P HGT 10 Evaluating the differences in inactivation kinetics upon solar irradiation of *Escherichia coli* with and without New Delhi metallo beta-lactamase gene
Nada Aljassim (Thuwal/Saudi Arabia)

P HGT 11 Integron diversity in bacterial communities of freshwater sediments is impacted by contamination
Justine Abella (Pau Cedex/France)

P HGT 12 Insect symbionts and plant pathogens share
Eva Novakova (Ceske Budejovice/Czech Republic)

P HGT 13 Viral-host interactions in the environment and their adaptive significance
Laura Sanguino (Ecully/France)

P HGT 14 The bovine plasmidome, a genetic hub for microbial genetic communication
Aya Brown Kav (Bet Dagan/Israel)

P HGT 15 Identifying large conjugative plasmids in draft genomes
Tue Kjærgaard Nielsen (Roskilde/Denmark)

P HGT 17 Comparative metamobilomics of rat-gut bacteria from pristine islands, rural areas and hospital sewers
Lars Hansen (Roskilde/Denmark)

P HGT 18 ComQXPA quorum sensing systems may not be unique to *Bacillus subtilis*: a census in prokaryotic genomes
Ines Mandic Mulec (Ljubljana/Slovenia)

P HGT 19 Horizontal gene transfer through plasmid transport: Heavy metal and antibiotic tolerance in bacteria
Erum Shoeb Nasir (Karachi/Pakistan)

P HGT 20 Bacterial community shifts and horizontal gene transfer assessment in silver stressed soils
Sotirios Vasileiadis (Mawson Lakes/Australia)

P HGT 21 Conjugative DNA Transfer in *E. coli* Isolates Increases Antibiotic Resistance in Urban Waterways
Krassimira Hristova (Milwaukee/United States)

P HGT 22 Urban wastewater treatment plants and dispersion of carbapenem resistant bacteria into the environment
Marcos Quintela-Baluja (Newcastle Upon Tyne/United Kingdom)

Constructed microbial communities as a tool in microbial ecology

P CMC 1 Continuous culturing as ideal model system to study the potential spread of antibiotic resistance in aquatic microbial communities
Gianluca Corno (Verbania/Italy)

P CMC 2 Understanding reactions of bacteria introduced into contaminated soil systems with the purpose of bioremediation
Jan Roelof van der Meer (Lausanne/Switzerland)

P CMC 3 Microbial ecology approach of anodic biofilms for the study of scaled-up microbial fuel cells
Agathe Paitier (Ecully/France)

P CMC 4 Unravelling the relationship between indigenous community diversity and success of bioaugmentation using synthetic microbial ecosystems
Johanna Vandermaesen (Leuven/Belgium)

P CMC 5 A new molecular tool designed to evaluate the potential-emissivity of nitrous oxide by microbial ecosystems
Anne Goubet (Antony/France)

P CMC 6 Construction of synthetic microbial consortia for the bio-conversion of pig slaughterhouse keratin wastes into high quality feed
Samuel Jacquiod (Copenhagen/Denmark)

P CMC 7 Construction of synthetic actinobacterial communities for oil-contaminated soil and water bioremediation
Maria Kuyukina (Perm/Russian Federation)

P CMC 8 Innovative washing solution based on *Lactococcus lactis*, nisin producing strain, and thyme essential oil at industrial level to improve safety and quality of minimally processed lamb's lettuce
Francesca Patrignani (Cesena/Italy)

P CMC 9 Towards an understanding of the sensitivity of pesticide degradation to losses of soil microbial diversity
Jessica Princivalle (Reading/United Kingdom)

P CMC 10 From Mouth to Model: improving methods for oral biofilm analysis
Martin Grube (Graz/Austria)

P CMC 11 Assessing the soil microbial interactome
Manupriyam Dubey (Lausanne/Switzerland)

P CMC 12 Studying the effect of increasing micro-predator and prey diversity on wastewater pathogen removal in a miniature membrane bioreactor system
Julia Johnke (Leipzig/Germany)

P CMC 13 P. *aeruginosa* PAO1 provides grazing resistance to sensitive strains in a multispecies biofilm
Henriette Lyng Røder (Copenhagen/Denmark)

P CMC 15 Ecology and evolution of kin recognition in *Bacillus subtilis*
Ines Mandic Mulec (Ljubljana/Slovenia)

P CMC 16 Temporal-niche partitioning of key species and their functions in a 14-year anaerobic benzene-degrading bioreactor
Ulisses Rocha (Amsterdam/The Netherlands)

P CMC 17 A novel high-throughput drip-flow system to grow autotrophic biofilms of contrasting diversities
Marta Kinnunen (Kongens Lyngby/Denmark)

P CMC 18 Microbial consortia bred from soil on different lignocellulosic substrates reveal distinct players acting in lignocellulose degradation
Maria Julia Brossi (Groningen/The Netherlands)

P CMC 19 Bacterial community structure changes during colonization and fruiting body production of oyster mushroom using a composted natural substrate
Balázs Vajna (Budapest/Hungary)

P CMC 20 Measuring patterns by geographical locations in marine metagenome Data using newly adopted genotyping by sequencing
Hoon Je Sung (Anseong/Republic of Korea)

P CMC 21 Dividing metabolic labor among microbial cells accelerates the consumption of substrates that produce growth-inhibiting intermediates
David Johnson (Dubendorf, Zurich/Switzerland)

P CMC 22 Combining microbial microcosms and mathematical models to enhance our quantitative understanding of diversity-disturbance relationships in ecology
Sean Gibbons (Chicago/United States)

P CMC 24 Taxonomic affiliation of esterase and lipase sequences of a metagenomic library constructed from oil-impacted mangrove sediment
Lucélia Cabral (Paulínia/Brazil)

P CMC 25 Arundo donax hydrolysates shape hydrogen-producing microbial community in dark fermentation processs
Anna Corsini (Milan/Italy)

P CMC 26 Enrichment and development of a microbial community capable of organic municipal solid waste degradation and ethanol production
Priscilla Carrillo-Barragan (Newcastle upon Tyne/United Kingdom)

P CMC 27 Screening of antibiotic producing actinomycetes from the sediments of undisturbed forest areas of Asella, Ethiopia and its hyper activity after mutation
Pakkianathan Ashokkumar (Sokoto/Nigeria)

P CMC 28 Responses of synthetic microbial communities perturbed in multiplexed continuous bioreactors as an ecologically-relevant proxy for microbial robustness prediction in natural and engineered ecosystems
F. Augellett (Louvain-la-Neuve/Belgium)

From microbial communities to single cells and back

P FMC 1 Bacterial and fungal organisms associated with dining tables in South East Nigeria, West Africa
Hope Okereke (Uturu/Nigeria)

P FMC 2 Aqueous two phase extraction of *Jonesia denitrificans* xylanase 6 in PEG 1000/phosphate system
Boucherba Nawel (Béjaia/Algeria)

P FMC 3 An antifungal peptide from actinobacteria (*Streptomyces* sp. TKJ2) Isolation and partial characterization
Messis Abdelaziz (Béjaia/Algeria)

P FMC 4 Magnetic in situ hybridization (MISH) combined to specifically adapted microfluidics as a complementary approach to metagenomics
David Royet (Ecully/France)

P FMC 5 Exploring diverse environments using metagenomics for novel natural products
Chiara Borsetto (Coventry/United Kingdom)

P FMC 6 Long-term cultivation of soil microorganisms in Nanoliter-Droplet Arrays
Johann Michael Koehler (Ilmenau/Germany)

P FMC 7 Metagenomic analysis of the acid mine drainage from gold mine in Western Siberia revealed novel bacterial lineage
Nikolai Ravin (Moscow/Russian Federation)

P FMC 8 Ecological diversity of the genus Acinetobacter and its role in genetic diversification and emergence of pathogenicity
Marc Garcia-Garcera (Paris/France)

P FMC 9 Combination of DNA stable isotope probing with cultivation-dependent methods for the comprehensive evaluation of the biodegradation potential of cis-1,2-dichloroethene
Serena Fraraccio (Prague/Czech Republic)

P FMC 10 Biosynthesis of microcystin in *Fischerella* sp. strain CENA161: Identification of the mcy gene cluster and toxin variants
Karina Heck (Piracicaba/Brazil, Helsinki/Finland)

P FMC 11 Ecosystem structure and potential functional diversity in microbiota-adapted lignocellulosic biomass
Valeria Ventorino (Portici/Italy)

P FMC 12 Transcriptional profile of *Corynebacterium pseudotuberculosis* 258 biovar *equi* submitted to conditions stress
Artur Silva (Belém/Brazil)

P FMC 14 Draft genome of the nitrogen-fixing cyanobacteria *Nostoc sp.* CENA 21, isolated from soil sample nearby Solimões river at Amazon, Brazil
Maria Paula Cruz Schneider (Belém/Brazil)

P FMC 15 Aqueous two phase extraction of *Jonesia denitrificans* xylanase 6 in PEG 1000/phosphate system.
Boucherba Nawel (Béjaia/Algeria)

P FMC 16 Linking alpha-diversity estimates between community fingerprinting and high-throughput 16S rRNA gene amplicon sequencing
Stilianos Fodelianakis (Thuwal/Saudi Arabia)

P FMC 17 Predator-prey networks in wastewater treatment plants: A potential way to improve pathogen removal
Julia Johnke (Leipzig/Germany)

P FMC 18 *Staphylocoagulase*, an exploitable intra- and inter-specific public good
Urvish Trivedi (Copenhagen/Denmark)

Gradients in conventional and extreme habitats

P GCEH 1 Diet determines bacterial diversity and community structure in Bornean pitcher plants
Wiebke Sickel (Würzburg/Germany)

P GCEH 2 Biogeography of the western Swiss Alps: teasing apart the effect of the environment on the soil bacterial communities.
Erika Yashiro (Lausanne/Switzerland)

P GCEH 3 Gradient of soil organic matter quality affects microbial community function and activity in forest soils
Petr Baldrian (Prague/Czech Republic)

P GCEH 4 Plastic debris: a distinct niche in the marine environment
Caroline De Tender (Merelbeke Ghent/Belgium)

P GCEH 5 Microbial community structure and function vertical distribution in snowpack over sea ice from Greenlandic fjord
Lorrie Maccario(Lyon/France)

P GCEH 6 Halophilic Bacteria and Archaea of Internal Saltmarsh in Saudi Arabia
Majed Albokari (Riyadh/Saudi Arabia)

P GCEH 7 A microfluidic chip to measure bacterial chemotaxis
Clemence Roggo (Lausanne/Switzerland)

P GCEH 9 Bacterial diversity in freshwater polar environments of Svalbard: bioprospecting for novel low temperature active hydrolases
Panayota Stathopoulou (Agrinio/Greece)

P GCEH 10 Adaptation of *Methylocystis* sp. strain SC2 to salt stress revealed by global transcriptome analysis
Dongfei Han (Marburg/Germany)

P GCEH 11 Microbial communities in the CO2-dominated active fault zone in NW Bohemia
Mashal Alawi (Potsdam/Germany)

P GCEH 12 Coping with copper: Soil active bacterial communities following 100 years of copper contamination
Inês Nunes (Copenhagen/Denmark)

P GCEH 13 Metagenomic analysis of microbial community structure of the acid mine drainage from Transbaikal Area, Russia
Vitaly Kadnikov (Moscow/Russian Federation)

P GCEH 14 Biogeographical distribution of Class I integron as bioindicators for freshwater systems
Luigimaria Borruso (Bolzano/Italy)

P GCEH 15 The genetic structure and characterisation of environmentally-associated *Escherichia coli* from water catchments in Eastern Australia.
Samantha Burn (Canberra/Australia)

P GCEH 16 Bacterial diversity of Paraguaçú: a river in Brazilian Semiarid biome
Ricardo Henrique Kruger (Brasília/Brazil)

P GCEH 17 Dynamics of bacterial communities in cryoconite holes of temperate glaciers
Isabella Gandolfi (Milan/Italy)

P GCEH 18 Spatiotemporal patterns and adaptation mechanisms of microbial communities in alkaline soda pans
Attila Szabó (Budapest/Hungary)

P GCEH 19 Comparison in two water microbial populations of iron mining region disabled by cultivation techniques and potential of enzymes production
Milena Tavares Lima (Jaboticabal/Brazil)

P GCEH 21 Increasing salt concentration causes shift to the dominance of marine picocyanobacterial taxa in continental waters
Anikó Mentes (Budapest/Hungary)

P GCEH 22 Microbial communities response to PAH contamination in coastal sediments
Mathilde Jeanbille (Pau/France)

P GCEH 23 Applying unconventional techniques for the laboratory cultivation of new bacterial strains
Tamas Felfoldi (Budapest, Miercurea Ciuc/Hungary)

P GCEH 24 Bacterial community succession in wood decomposition
Anna Kielak (Wageningen/The Netherlands)

P GCEH 25 The rise and fall of the rare biosphere: soil bacteria response to flash floods and desiccation in the desert
Osnat Gillor (Midreshet Ben Gurion/Israel)

P GCEH 26 Salinity and temperature effects on crude oil methanogenic biodegradation in river Tyne microbiota
Isabel Natalia Sierra-Garcia
(Campinas/Brazil, Newcastle upon Tyne/United Kingdom)

P GCEH 27 Drinking water microbiome: from groundwater to the tap
Antonia Bruno (Milan/Italy)

P GCEH 28 Impact of local and global metal contaminations on microbial communities of Pyrenean lakes
Jean-Christophe Auguet (Pau/France)

P GCEH 29 Soft coal mining and mineral leaching – bacterial life in slag deposits
Carl-Eric Wegner (Marburg/Germany)

P GCEH 30 The effect of fish farming on sediment microbial community
Antti Karkman (Helsinki/Finland)

P GCEH 31 A framework to understand the ecological mechanisms mediating microbial community assembly along successions
Francisco Dini-Andreote (Groningen/The Netherlands)

P GCEH 32 Bacterial diversity and chemical pollutants investigation in ice core of Campbell Glacier in Northern Victoria Land in Antarctica
Fabrizio Cappa (Piacenza/Italy)

P GCEH 33 Evidence for shift from *Acidobacteria* to Proteobacteria dominance in soil profile of boreal acid sulphate soils in Finland
Alica Chronakova (Ceske Budejovice/Czech Republic)

P GCEH 34 Large scale spatial analysis of bacterial communities in lake sediments, the role of physico-chemical parameters, spatial distance, land cover and tropical storms
Jean-Sebastien Beaulne (Ecully/France)

P GCEH 36 Spatial pattern of bacterial diversity in a site with mixed and uneven contamination, and assessment of rhizoremediation potential
Francesca Mapelli (Milan/Italy)

P GCEH 37 Diversity and small-scale distribution of microbial communities in patterned grounds
Ilaria Mania (Grugliasco/Italy)

P GCEH 38 Changes in bacterioplankton community and carbon cycling rates along the subtropical frontal zone off New Zealand suggest preferences in metabolic lifestyles associated to different water masses
Sergio Morales (Dunedin/New Zealand)

P GCEH 39 Cyanobacteria, too small to sink? Relative and absolute contribution of cyanobacteria to the carbon export in the Sargasso Sea
Francesca De Martini (Tempe/United States)

P GCEH 40 Influence of environmental pH on thaumarchaeal diversification
Cecile Gubry-Rangin (Aberdeen/United Kingdom)

P GCEH 41 Effects of Saharan dust storms on the diversity and composition of the microbiota in mountain glacier snow
Ermanno Federici (Perugia/Italy)

Novel biodegradation pathways along spatial gradients

P NOV 1 New antibiotic resistance genes from a soil metagenomic library and their unexpected application in bioremediation processes.
Ricardo Kruger (Brasilia/Brazil)

P NOV 2 Purification and characterization of a novel carbofuran hydrolase from the carbofuran mineralizing Novosphingobium sp. KN65.2
Basak Ozturk (Leuven/Belgium)

P NOV 3 Looking for a perfect microorganism decomposing sodium dodecyl sulfate: environmental and genomic study
Ewa Furmańczyk (Warsaw/Poland)

P NOV 4 Functional redundancy of multicomponent monooxygenases extend catabolic versatility in phenol- and toluene-degrading bacteria
Maarja Grünbach (Tartu/Estonia)

P NOV 5 Map of the aromatic catabolic potential in the *Roseobacter* lineage
Catalina María Alejandro-Marín (Palma de Mallorca/Spain)

P NOV 6 Chlorobenzene Isotopic Fingerprinting analysis and microbial genetic profiling of natural consortia from a contaminated aquifer
Giovanna Carpani (San Donato Milanese/Italy)

P NOV 7 Microbiological processes for the degradation of biodiesel in groundwater
Franciele Fedrizzi (Lyon/France)

P NOV 8 Microbial diversity and function during different bioremediation strategies of diesel-polluted soil
Thibaut Masy (Ecully, Gembloux/France, Liège/Belgium)

P NOV 9 Biostimulation of anaerobic biodegradation by iron reduction processes in groundwater contaminated with a diesel/biodiesel blend (B20)
Juliana B. Muller (Ecully/France, Florianópolis/Brazil)

P NOV 10 Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) degradation ability in Fosmid library from oil-impacted mangrove sediment
Sanderson Tarciso Pereira de Sousa (Paulínia/Brazil)

P NOV 11 Microbial community responses to contamination in mangrove sediment as revealed by metatranscriptomics
Lucélia Cabral (Campinas/Brazil)

P NOV 12 Deciphering the roles of the members of a bacterial consortium in the degradation of thiabendazole: combining SIP-DGGE with meta-omics
Dimitrios Karpouzas (Larissa/Greece)

P NOV 13 Nickel and Copper biosorption by EPS producing *Ensifer adhaerens* strain As3-5a
Besma Ettoumi (Milan/Italy)

P NOV 14 Assessment of anaerobic biodegradation of polyvinylchloride by enriched marine communities
Noura Raddadi (Bologna/Italy)

P NOV 15 Inferring oxybenzone biodegradation at low concentrations through bacterial biomass measurements
Estelle Clerc (Lausanne/Switzerland)

The food to gut microbial continuity

P FOOD 1 New insights into the ecology of supralittoral sediments detritivors from the analysis of gut microbiota of talitrid amphipods
Khaled Farag A Abdelrhman (Florence/Italy)

P FOOD 2 The microbiota from choanae of selected free-living birds species
Tjaša Matjašič (Maribor/Slovenia)

P FOOD 3 Differences of bacterial community composition between caecum and colon of rats after long term dietary intervention.
Heli Jaime Barron Pastor (Canberra/Australia, Lima/Peru)

P FOOD 4 *Escherichia coli* in poultry meat: prevalence, abundance and phylogenetic profiles
Belinda Vangchhia (Canberra/Australia)

P FOOD 5 Effects of diet and predation on fish gut microbiota
Yinghua Zha (Uppsala/Sweden)

P FOOD 6 From gut to food and back to gut: bacterial diversity in animal casings used in the production of dry fermented sausages
Edoardo Puglisi (Piacenza/Italy)

P FOOD 7 Influence of food and environmental factors on human gut bacterial community networks in early childhood
Marion Engel (Neuherberg/Germany)

P FOOD 8 Fitness landscape of the world's most famous operon in a natural environment
Catarina Pinto (Oeiras/Portugal)

P FOOD 9 Unraveling autism: a crowdsourced clinical trial to genotype and sequence the microbiome of autistic children and their siblings
Maude M. David (Stanford/United States)

P FOOD 10 Traditional Italian dairy products, a flavorful source of naturally occurring bacteria with beneficial effects on health
Franca Rossi (Verona, Campobasso/Italy)

P FOOD 11 Study on the effect of piliation on the colonisation ability of *Lactobacillus rhamnosus* GG
Pia Rasinkangas (Helsinki/Finland)

P FOOD 12 Effects of disodium fumarate on ruminal bacterial communities of early weaning lambs
Wang Xinfeng (Shihezi/Republic of China)

Late Abstracts

P LATE 1 Differential physiological profiles at the soil particle size level explain the differences between no-till and conventional tillage agricultural managments of the same soil
Luis Wall (Bernal/Argentina)

P LATE 2 Situation of genes involved in pathogenicity of potato raised and deep pitted inducing scab bacterium
Gholam Khodakaramian (Hamedan/Islamic Republic of Iran)

P LATE 3 Drug metabolism of human gut bacteria
Martina Klünemann (Heidelberg/Germany)

P LATE 4 Isolation and proteogenomic analysis of a Sphingomonas haloaromaticamans strain able to degrade the fungicide ortho-phenylphenol used in the fruit-packaging industry
Chiara Perruchon (Larissa/Greece)

P LATE 5 Bacterial diversity in sugarcane filter cake following incomplete composting process along 60 days
Luciano Takeshi Kishi (Jaboticabal/Brazil)

P LATE 6 Diversity and rehabilitation of prokaryotic and fungal communities of European soil crusts
Martin Grube (Graz/Austria)

P LATE 7 Land management and seasonal changes impact on chernozems prokaryotic soil microbial community
Mikhail Semenov (Moscow/Russian Federation)

P LATE 8 Microbiome taxonomic structure and diversity of two interconnected semi-arid soils of Caspian Depression
Mikhail Semenov (Moscow/Russian Federation)

P LATE 9 Lichen-associated bacterial communities: studies of bioconversion of lichen metabolites
Martin Grube (Graz/Austria)

P LATE 10 The crystal structure of phthalate dioxygenase reductase protein from a typical ultramicrobacteria PAE-UM
Dan Ma (Tianjin/Republic of China)

P LATE 11 Comparison of bacterial community diversity in sugarcane vinasse
Noriko Cassman (Wageningen/The Netherlands)

P LATE 12 Directional and non-directional β-diversity of fungi along the alpine landscape
Eric Pinto-Figueroa (Lausanne/Switzerland)

P LATE 13 Bacteriophages as natural evolutionary enhancers in bacteria
Alexander Rakin (Munich/Germany)

P LATE 14 Comparing the gut microbiota of two communities from Amazon region with the Southeastern in Rio de Janeiro, Brazil
Eder Soares Pires (Rio de Janeiro/Brazil)

P LATE 15 Biodegradation of selected plastics by psammon bacteria occupying the sand of a recreational marine beach
Katarzyna Koszycka (Gdansk/Poland)

P LATE 16 Biogeography of soil nitrogen fixing bacteria in the western Swiss Alps
Davide Ciccarese (Lausanne/Switzerland)

P LATE 17 The Transferome analysis allows the inference of the current and paleo-environments
Sandrine Pinos (Marseille/France)

P LATE 18 Potential of an actinomycete, Micromonospora halophytica, to enhance tolerance of tomato (Lycopersicon esculentum) to soil salinity through production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase
Khaled El-Tarabily (Al-Ain/United Arab Emirates)

P LATE 19 Promotion of growth of Salicornia bigelovii in a calcareous saline soil by phosphate-solubilizing halophilic actinomycetes
Khaled El-Tarabily (Al-Ain/Unites Arab Emirates)

P LATE 20 Invading and getting invaded: a view from both sides
Nico Boon (Ghent/Belgium)

P LATE 21 Identification and genome analysis of a novel Pseudomonas veronii isolate with biocontrol activity against the nematode Xiphinema index
Fabiola Altimira (Valparaiso/Chile)

P LATE 22 Effect of the soil types and the plant species on the rhizosphere competence and biocontrol activity of Pseudomonas jessenii RU47 at the field scale
Rita Grosch (Großbeeren/Germany)

P LATE 23 Effect of Microbial extracts of Algerian soil against Culex pipiens (Diptera: Culicidae)
A. Aouati (Constantine/Algeria)

P LATE 24 Bacterial biodiversity in a chemolytotrophic cave
P. García-Fraile (Prague/Czech Republic)

Abstracts

KN 1

Biological soil crusts: ancient and widespread microbial communities

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In areas where plant cover is restricted and plant litter scarce, light reaches unimpeded the soil surface, creating a habitat for microbial phototrophs. These in turn support plant-independent complex soil communities known as biological soil crusts (BSCs) because of the physical effect they have on soil surface consolidation. While relatively inconspicuous to microbiologists until relatively recently, much progress has been made in the study of BSCs in the last decade. It is now known that they account for a sizeable fraction of the global microbial phototrophic biomass, and that they are globally relevant contributors to the nitrogen cycle. At the local scale, they become important ecosystem components, particularly in arid lands, as agents of C and N fertility, as well as in erosion control. But BSCs face serious challenges from a variety of human activities, chiefly from trampling by cattle and off-road vehicles. Consequently, the enhancement and restoration of natural BSC cover is emerging as a promising means for sustainable management of arid soils, with significant efforts are being currently conducted in China, Europe and the US. Microbial growth in BSCs occurs in spite of a pulsed regime of activity imposed by the episodic nature of water availability, and in a habitat that can be characterized as harsh in more respects than one. BSC microbes present fascinating adaptations to a crusty life that include morphological, biochemical and behavioural responses, from the synthesis of UV sunscreens, the use of hydrotaxis and phototaxis, bundle-formation, and the secretion of abundant EPS. Recently presented fossil evidence suggests that BSC-like communities have colonized Earth's terrestrial sediments since at least the mid Proterozoic (1.3 billion years ago), and may represent the major type of terrestrial ecosystem before the advent of higher plants in the Devonian, some 0.4 billion years ago. In this presentation, I review some of my group's findings regarding the biology of soils crusts, in which we used a variety of approaches that combines traditional microbiology and omics-based microbial ecology techniques with methodologies from geoscience.

KN 2

Microbiome-assisted protection of plant roots

G. Berg[1]

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The importance of microbial root inhabitants for plant growth and health has been recognized already 100 years ago. Since that time, much has been learned about microorganisms and their close symbiotic relationship with plants (Berg *et al.* 2015). Comparable to humans and other eukaryotic hosts, plants also may be realized as meta-organism. These advances in knowledge were driven by both "omics"-technologies guided by next-generation sequencing and microscopic insights. Root-associated microbes can help plants fend off disease, stimulate growth, occupy space that would otherwise be taken up by pathogens, promote stress resistance, and influence crop yield and quality. Therefore, the root microbiome is a key determinant of plant health and productivity. Root microbiome discoveries could fuel progress in sustainable agriculture, such as the development of microbial inoculants as biofertilizers, biocontrol, or stress protection products (Berg 2009). Although we recognize a growing market for these bio-products, they still have their problems, e.g., short shelf-life, inconsistent effects under field conditions, and risk predictions. The application of "omics"-technologies has allowed for an enormous progression in the development of so-called next-generation bio-products (Berg *et al*. 2013). New tools may have an impact on (i) the detection of new bio-resources for biocontrol and plant growth promoting agents, (ii) the optimization of fermentation and formulation processes for biologicals, (iii) stabilization of the biocontrol effect under field conditions and (iv) risk assessment studies for biotechnological applications. Advances in these aspects could open new perspectives for sustainable agriculture by the development of high impact next-generation bio-products.

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KN 3
Diversity and function of Arctic snow and ice
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The cryosphere is a fundamental control of the physical, biological and social environment over a large part of the Earth's surface. It is an integrator of climate variability, providing visible signals of change, and yet, it is also an actor, intrinsically involved in global cooling through a number of feedback mechanisms. The cryosphere environment is not only undergoing changes due to climate shifts, but also due to long-range transport of contaminants and increased human activity. Among the most critical, yet under-studied components of the cyrosphere are the microbial communities inhabiting the ice and snow. Due to the cold conditions and the limited supply of liquid water, snow and ice have long been considered as entrapment and storage systems for microorganisms, nutrients, soluble inorganic and organic matter and contaminants delivered by wet and dry deposition. However, recent reports have shown that these environments are far from inert, and constitute habitats for microorganisms that are able to transform, metabolize and alter biogeochemical cycling. Here, we present recent findings on microbial communities from Arctic ice and snow and focus on how they function in a changing climate. In order to predict how ecological processes will evolve as a function of global change, knowledge concerning the populations that participate in each process, and their physiology and function is essential. In addition, the changes in their relative abundance, activity and community structure due to these constantly changing environmental conditions is critical.

KN 4
Arbuscular Mycorrhizal fungi and their endobacteria: a symbiotic microbiota
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[1]*University of Torino, Department of Life Science and Systems Biology, Torino, Italy*

Being present in the rhizosphere and in plant tissues as obligate symbionts, Arbuscular Mycorrhizal (AM) fungi are important members of the plant microbiota. As beneficial microbes, they play a key role in nutrient cycling, and boost plant growth, improving water and mineral nutrient uptake. They also provide protection against biotic and abiotic stresses. For these reasons, AM fungi are currently acknowledged as a driving force for plant evolution. By contrast, the fact that many of them contain endobacteria inside their cytoplasm is much less known.
By using a combination of molecular, phylogenetic and cell analysis we have found that distinct types of endobacteria may coexist in a single AM fungal spore, showing that AM fungi host a so far poorly known intracellular bacterial microbiota. The genome sequencing of one endobacterial type (*Candidatus* Glomeribacter gigasporarum) has revealed a strong genome reduction when compared to the free-living related taxa: the endobacterium depends on its host for carbon, phosphorus and nitrogen supply; it also expresses type III secretion systems, synthesizes vitamin B12, and toxin-resistance molecules, which may contribute to the fungal host's ecological fitness.
To understand the impact of the bacterium on the fungus, a cured line of the fungus *Gigaspora margarita* was created, where a decrease on the spore number production has been observed. Due to the limited number of genomic data on AM fungi, which at the moment are limited to *Rhizophagus irregularis,* a transcriptomic RNA-seq analysis was performed to get a gene catalogue for *G. margarita*, and to compare its transcriptomic profile with and without the endobacteria. We find that the endobacteria and the fungus cooperate, raising the fungal bioenergetic capacity, controlling oxidative stress, and regulating the intracellular calcium concentration. We hypothesise that such mechanisms have important consequences for AM fungi, which produce more spores, leading to more events of plant colonization.
The discovery of such a tripartite symbiosis unravels a complex network of interdomain interactions, which are expected to have a previously unrecognized ecological impact.

KN 5
Gendercide symbionts and hopeful symbiotic monsters, in natural and man-made ecosystems
C. Bandi
No abstract submitted.

KN 6
Gut-microbe symbiosis and dysbiosis: A view from Drosophila
W.- J. Lee[1]
[1]Seoul National University, National Creative Research Initiative Center for SymbioSystem, and Department of Biological Science, Seoul, Korea, Republic of

Gut microbiota is found in virtually any animals, from invertebrates to vertebrates. It is now evident that gut microbiota directly influences a variety of aspects in animal physiology such as immunity, development, and metabolism. However, the exact molecular mechanisms by which gut microbiota achieves the host physiological homeostasis are largely unexploited. Here I will present and discuss recent discoveries regarding the molecular dialogues between bacteria and animals, using a genetic *Drosophila* model organism. Specifically, I will introduce how gut epithelia react to pathogens by using oxidant weapons, how beneficial gut bacteria influence host immunity and development, and how gut immunity distinguishes between beneficial commensal bacteria and life-threatening pathogens. Future studies in this direction in different invertebrate and vertebrate animal models will certainly provide a unique opportunity to better understand the evolutionarily conserved dialogue between prokaryotes and eukaryotes.

KN 7
The food-human axis: dietary habits and gut microbiota and metabolome
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A number of 10-100 trillion microbes populate the human intestine, with 100-fold more bacterial genes than human genes. The gut microbiota is 10-fold more than human cells, being subjected to variations due to body size, age, diseases and nutritional intake. Intestinal bacteria interact with each other and the host through metabolite production and substrate fermentation, which emphasizes why diet has a pivotal role in modifying human intestinal microbiota (1). The gut microbiota and metabolome was assessed in a large cohort of Italian individuals comparing habitual diets (omnivore, vegetarian and vegan). Dietary patterns and associated microbiota stratified individuals according to diet type. Consumption of vegetable-based diets was significantly associated with increased levels of faecal short-chain fatty acids, *Prevotella* and a number of fibre-degrading Firmicutes. Conversely, high urinary trimethylamine oxide levels were found in individuals with lower adherence to the Mediterranean diet (2). β-glucans, as an important dietary component, positively influenced the faecal microbiota and metabolome of healthy individuals during two months of diet intervention (daily intake of 3 g of barley β-glucans). Pyrosequencing data showed that Clostridiaceae (*Clostridium orbiscindens*, *Clostridium* sp. and *Roseburia hominis*) and *Ruminococcus* sp. increased, while other Firmicutes and *Fusobacteria* decreased. 2-Methyl-propanoic acid, acetic, butyric, hexanoic and propionic acids also markedly increased (3). Celiac disease (CD) is an increasing food intolerance, which inevitably affects the dietary habits. A gluten-free diet (GFD) lasting at least two years did not completely restore the microbiota and, consequently, the metabolome of CD children (4). Keeping excluded gluten, the GFD style had also repercussions. Saharawi CD children, following an African-style GFD for at least two years, were subjected to a change into an Italian-style GFD. The switch modified the previous imbalance to a new imbalance, altering the salivary type microbiota of individuals. The metabolic potential of the microbiota markedly changed (5). Stating that the exclusion of gluten-containing cereals from the diet somewhat altered the human microbiome and metabolome, gluten-free baked goods made with hydrolysed wheat flour have been manufactured and introduced into the CD market (6).

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KN 8
Multi-omics of the human gut microbiome
J. Jansson
No abstract submitted.

KN 9
Soil metagenomics and forensics
P. Simonet
No abstract submitted.

KN 10
Why Metagenomics?
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Metagenomics is one of the current fashionable methods in microbial ecology, although many of us do not actually produce genomes. Experimental and bioinformatic methods are moving towards the possible extraction of the genomes from different microorganisms in an ecosystem. Some genomes have been published and/or are underway. The numbers of these genomes is currently considerably less than that provided after the isolation and sequencing of microorganisms from the different ecosystems. Metagenomics avoids the bias of laboratory culture and instead has a plethora of other problems. Yet, high throughput sequencing (often called metagenomics) can be used without the post-sequencing construction of putative genomes to provide (quasi-) quantitative measurements of different genes and gene families related to our hypothesies, especially in dynamic and spatial relationships. The number of different genes that can be explored simultaneously far exceeds that reasonably produced by quantitative PCR. The accidental or incidental confusion about the relative meaning and applications of metagenomics and high throughput sequencing has clouded the importance of inexpensive sequencing for microbial ecology studies. The data provided by high throughput of genes and gene families often relates better to questions and hypotheses than the metagenomic production of putative genomes, although this may change in the future.

KN 11
Massive parallel amplicon sequencing as a means to reveal niche preferences and eco-physiological properties of uncultured microbial taxa
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Massive parallel sequencing of PCR amplicons obtained from directly extracted environmental DNA is increasingly applied to explore the diversity of microbial communities and their variability across temporal or spatial scales. Beyond that, comparison of microbial diversity from different environmental or experimental variants can indicate responses of specific taxa targeted by rRNA or functional gene analyses. For microbial community analyses from soil it is not unusual to compare communities each composed of more than 10,000 different taxa (OTUs at>97 % sequence identity), each with a particular abundance. For comparative community analyses, specific statistical procedures are required to filter out background-noise and to allow the distinction between false positive and significantly different OTUs. The identification of significantly different taxa may also call for particular experimental designs, e.g., with a larger number of replicates. In this short presentation we want to report on two examples in which we analysed prokaryotic communities from soil for the presence of specific taxa. In one study we searched for OTUs in pristine shrubland soil with sensitivity/tolerance in response to saline irrigation water for the cultivation of wheat. The other study concerns the detection of OTUs with preference for specific soil particle size fractions in agricultural soils and their responsiveness to long-term treatment with mineral and organic fertilizers.

KN 12
The microscale dynamics of ocean microbes
R. Stocker
No abstract submitted.

KN 13
"Omics" and microbial ecology – opening new windows of research
M. Ferrer Martinez
No abstract submitted.

KN 14
Diverse contributions of IncP-1 plasmids to bacterial adaptation in the environment
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Horizontal gene transfer (HGT) is recognized as a major force contributing to bacterial adaptation and diversification. Plasmid-mediated HGT is assumed to be essential for a relatively rapid response to changing environmental conditions. IncP-1 plasmids were first described in the 1970's from clinical isolates (RP4, R751). Only later they were also discovered in isolates from contaminated soils (e.g. pJP4). The biochemistry of plasmid backbone encoded functions such as replication, maintenance and transfer were studied in great detail. IncP-1 plasmids were shown to transfer and replicate in a wide range of gram-negative hosts and the transfer range is even broader. They efficiently transfer, e.g., in soils and in the phytosphere, and many of the recently described IncP-1 plasmids were exogenously captured into recipients. But the role and ecology of IncP-1 plasmids were only studied in more depth after tools to monitor IncP-1 plasmids had become available. Nowadays, the complete sequences of 46 IncP-1 plasmids are available and at least seven subgroups are described. These sequences were not only essential for unravelling their diversity but they were also the basis for developing primers and probes. In this talk an overview is given of how the use of cultivation-independent methods provided insights into the dissemination, diversity and importance of IncP-1 plasmids in different ecosystems. Hot spots of IncP-1 plasmid occurrence are not only sewage, manure, digestates, contaminated soils and sediments; more recently IncP-1 plasmids were also found to be enriched in the mycosphere or in the rhizosphere of lettuce grown in unpolluted soils. This was surprising as several studies showed a correlation between IncP-1 abundance and pollution. In microcosms we could show that linuron application significantly increased the abundance of IncP-1 plasmids confirming observations made in the field. Besides their contribution to biofilm formation, an enormous range of accessory genes encoding traits ranging from antibiotic, metal and disinfectants resistance to degradative genes might explain the selective advantage of IncP-1 plasmids to their hosts. In addition, capturing IncP-1 plasmids with complete degradative pathways might be of biotechnological interest.

KN 15
Ecological concepts for anaerobic degradation of aromatic hydrocarbons in groundwater and oil reservoirs
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Despite decades of groundwater research the process understanding why contaminants are microbially degraded or not is still in its infancy and bioremediation actions often rely on trial and error treatments of black box approaches. In order to come to knowledge-based bioremediation approaches there is a great need for a next level of ecological principles and profound generic understanding of bottlenecks of biodegradation in the subsurface which goes beyond description of site situations or simple addition of nutrients etc.
We will present the latest developments of ecological concepts for biodegradation in groundwater and in oil reservoirs. Recent advances will be presented concerning the biodegradation pathways of polycyclic aromatic hydrocarbons such as naphthalene. The results from biochemical and genetic studies are used to demonstrate biodegradation in the field. Limitations for biodegradation in the subsurface such as the plume fringe concept and temporal fluctuations will be presented based on high resolution monitoring of geochemical gradients, microbial communities, and activities in the field. Such

concepts will be discussed in the context of oil reservoirs where we discovered a new habitat for biodegradation, microbial life in small water droplets enclosed in oil.

KN 16

Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities

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Many microbial ecologists have described the composition of microbial communities in a plenitude of environments, which has greatly improved our basic understanding of microorganisms and ecosystems. However, the factors and processes that influence the behaviour and functionality of an ecosystem largely remain black boxes when using conventional approaches. Therefore, synthetic microbial ecology has gained a lot of interest in the last few years. Because of their reduced complexity and increased controllability, synthetic communities are often preferred over complex communities to examine ecological theories. They limit the factors that influence the microbial community to a minimum, allowing their management and identifying specific community responses. However, besides their use for basic research, synthetic ecosystems also found their way towards different applications, like industrial fermentation and bioremediation

Oral presentations

O HGT 1

Selection for horizontal gene transfer shapes the social game between honest and eavesdroppers in constructed communities of *Bacillus subtilis*

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Introduction: When undertaking costly metabolic investments, bacteria rely on self-produced chemical signals that carry basic information about surroundings (i.e. local cell density or mass transfer) using quorum sensing (QS). Reliability of chemical information can be threatened by spread of eavesdroppers which by avoiding signal production provide false data on population size/density. What then constrains signalling and prevents eavesdroppers (signal null mutants) from spreading?
Objectives: By using a Gram positive bacterium *B. subtilis* and its major quorum sensing system that controls genetic competence for transformation as the model we aimed to address the social game between eavesdroppers (signalling mutants) and honest (signal proficient strains) and through this unravel the mechanisms that shape the stability of QS dependent cooperation.
Methods: We applied two types of competition assays: a) traditional growth competition assay where two strains (signal deficient and QS proficient) compete for limited nutrients, b) competition under additional antibiotics stress, where the two strains also need to develop competence for transformation and compete for extracellular DNA encoding the antibiotic resistance genes.
Results: We discovered that signal production negatively regulates the QS response in *B. subtilis.* This manifests in increased production of surfactin and increased competence for transformation in eavesdroppers. This imprudent response is associated with fitness costs for signal deficient strains under limitation for nutrients. Paradoxically, under antibiotic stress the over responsive mutants survive better than QS proficient strains because they take up more exogenous DNA and thus more selective traits needed for survival.
Conclusion: Evolutionary stability of signalling in *B. subtilis* can be explained by direct benefits, because the signal serves as a coercion to non-senders while the sender is equipped with the negative feedback regulation of the QS response. However, under antibiotic stress coupling low fitness with high recombination rate promotes survival of eavesdroppers. The work brings novel insights into intricate molecular mechanisms that shape the social game between honest and eavesdroppers in constructed communities of *B. subtilis*

O HGT 2

Bistable Integrative and Conjugative Element transfer in *Pseudomonas* is controlled by local nutrient levels

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Integrative and Conjugative Elements (ICEs) are mobile DNA, which are normally integrated in the host genome, but seldomly excise, form a circular intermediate, and conjugate to recipient cells. Here, they re-integrate into the genome at one or more specific sites. In an ICE model called ICE*clc*, originally discovered in *Pseudomonas knackmussii* B13, a subpopulation of 3 to 5% of cells in stationary phase becomes "transfer competent" (tc) for conjugation of ICE*clc.* The fate of individual cells that activate the ICE*clc* tc pathway is poory understood. Here we focus on the development of new tools which enable the study of ICE*clc* excision and transfer in real-time at the single cell level.
ICE*clc* variants were created that carry the *egfp* gene downstream of the *intB13* gene for integrase. Because ICE*clc* excision and recombination at the ends causes replacement of the promoter upstream of *intB13,* we expected that cells in which ICE*clc* is excised would be distinguishable by their EGFP expression. Specific recipients were constructed, which would "light up" when ICE*clc* integrates in their genome. Time-lapse microscopy of individual *Pseudomonas putida* cells carrying such ICE*clc* indicated three different EGFP expression levels. Further time-lapse microscopy experiments of *P. putida* donor and recipient cells indicated that the highest EGFP levels in donors was associated with cells actually transferring ICE*clc*. In contrast, the lowest EGFP expression occurred in cells in which ICE*clc* remains silent, and intermediate EGFP expression was found for cells which start the tc pathway. Interestingly, therefore, donor cells which activate ICE*clc* in stationary phase do not immediately excise it. Probably, only tc cells having access to sufficient nutrient are able to engage ICE*clc* excision, while most tc cells depleted in nutrient are not. Our results thus illustrate the unique steps in ICE transfer dependent in a broader ecological context. Fig. 1. ICE*clc* transfer observed in real time. In green, tc cells; in red, recipient cells.

Fig. 2. ICE*clc* transfer observed in real time, 5 hours later. In green, tc cells; in red, recipient cells; in yellow, transconjugants.

Figure 1

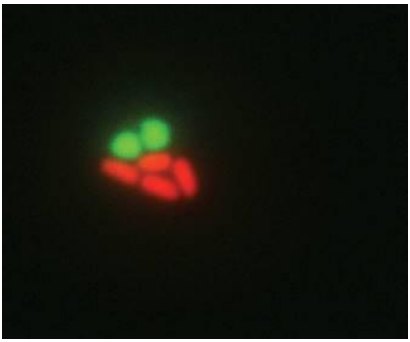
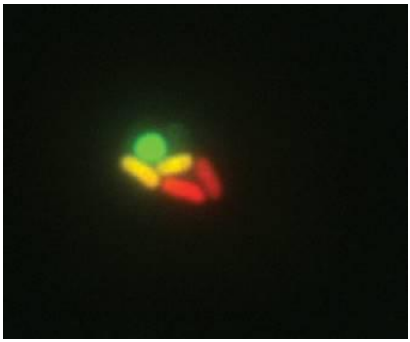


Figure 2



O HGT 3

c-di-GMP related genes are common on plasmids - a comparative analysis

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In most bacteria, the secondary messenger c-di-GMP is central for facilitating a behavioral response to different environmental clues. Specifically the transition between biofilm and motility behaviors has been linked to the intracellular lever of c-di-GMP in many different bacteria. High c-di-GMP levels typically induce biofilm phenotypes while low levels induce motile phenotypes. Here we focused mainly on two types of proteins, namelydiguanylatecyclases (DGCs) andphosphodiesterases (PDEs), which are responsible for synthesizing and degrading c-di-GMP, respectively.
We show that genes associated with the response and turnover of c-di-GMP are much more commonly found on plasmids than hitherto imagined, and that the number of such genes seemingly are higher per ORF on these plasmids compared to chromosomes. Degenerate versions of the active sites of both DGCs and PDEs have been shown to function as sensor-domains that can bind c-di-GMP. Interestingly, our comparative analysis suggests that there is a general difference in the distribution of genes that encode putative catalytic and sensor proteins between chromosomes and plasmids.
As proof of concept we illustrate that a plasmid encoded DGC and PDE, predicted to be catalytic active did indeed change the host biofilm phenotype *in vitro* in various different species of *Enterobacteriaceae*.
Our findings provide a strong link between horizontal gene transfer and the adaptation of basic bacterial behavior and illustrate that mobile genetic elements may impose specific phenotypes via a core regulatory system. We believe that these findings are best understood in light of theories on “genomic conflicts” and “selfish genes”.

O HGT 4

Metal stress modulates the immediate plasmid uptake potential of soil microbes

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Plasmid transfer is considered an important adaptive mechanism that can provide bacteria, among others, with resistance determinants against many environmental insults. However, it is not known if environmental stress itself can directly stimulate this process. Metal cations tend to accumulate in many soils due to agricultural practices such as manure fertilization and can be the source of significant stress exposure to soil microbial communities.
We, therefore, evaluated if imposing a metal stress alters a soil community's permissiveness towards broad host range plasmids and if such a potential stress response would be general or metal specific. Additionally we aimed to identify those bacterial taxa that respond to stress by increasing their plasmid uptake.
Using a ^{3}H-leucine incorporation approach, we defined 20% and 50% inhibition concentrations of 5 metals (Cu, Ni, Cd, Zn, As) for a reference soil microbial community. An *mCherry*-tagged donor carrying the zygotically inducible *gfp*-tagged plasmid pKJK5 was mated with a soil microbial community under these prescribed metal stresses. Transconjugants were quantified and isolated using advanced microscopy and fluorescence activated cell sorting. Sorted transconjugants as well as the corresponding stressed recipient communities were analyzed with 16S rRNA gene based amplicon sequencing. Ultimately we wished to identify whether metal stress responded in a change in the diversity of the community and in its transconjugal pools.
The imposed metal stress lowered the plasmid transfer frequency. The intensity of this effect was metal specific and not due to different growth inhibition of the recipient community, because the exposure to metals was normalized to similar inhibition levels.
Our results revealed a modulation of the phylogenetic composition of the transconjugal pools for the heavy metals Nickel and Cadmium, while Arsenic exposure had no effect on the observed transconjugal pools. The stress associated changes in β-diversity among transconjugal pools were metal specific and could not be directly explained by the changes in the recipient communities observed for the corresponding metal stresses. Our results therefore indicate a metal specific stress response and we were able to identify taxa within the soil microbial community that react to stress by increased plasmid uptake.

Poster presentations

P HGT 1

Mutagenic processes in environmental strains of pseudomonads

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In recent decades many researchers have successfully isolated bacteria capable of degrading compounds previously considered non- degradable in the nature. Most of these strains have been isolated from heavily polluted sites. Living in harsh environmental conditions can cause different problems for bacteria and is potentially mutagenic. We have hypothesized that exposure to toxic aromatic compounds forces bacteria with higher mutation frequency (mutator phenotype) to arise and through it speed up the evolution of new catabolic pathways. In this work we compared mutation rate in bacterial strains isolated from polluted and non-polluted sites in Estonia (CELMS collection). Studied strains were selected for their ability to degrade alkanes or different kind of aromatic compounds.
Among the 64 analyzed strains only a few of them exhibited significantly higher spontaneous mutation rate (based on the frequency of appearance of rifampicin resistant mutants) in comparison to our laboratory reference strain *Pseudomonas putida* PaW85. Furthermore, we did not find any strains with hypermutator phenotype typical for DNA mismatch repair defective bacteria. Most of the studied strains had similar or even lower mutation rate than PaW85. These results contrast to the finding that hypermutators are overrepresented in populations of pathogenic bacteria during chronic infections. The fact that we did not find any strains with high mutator phenotype even from heavily polluted sites does not necessarily mean that the evolution of new catabolic pathways is not connected with it. On the contrary, it may just show that high mutation frequency in a longer time scale is burdening for bacteria and it is beneficial to keep the mutational frequency at optimal level. Additionally we analyze the possibility of induced mutagenesis through the involvement of error-prone DNA polymerases. Further studies are in progress to examine dynamics of mutator phenotype in populations of environmental bacteria.

P HGT 2

Plasmid pP32BP2 of *Psychobacter* sp. DAB_AL32B and its role in host adaption to extreme arctic environment

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Introduction: Psychrophiles are a group of cold-adapted bacteria which live in permanently cold environments. These microorganisms developed specific attributes to tolerate such extreme conditions. Ability to survive may be achieved by the incorporation of various mobile genetic elements, plasmids in particular, which are known to be the main players in horizontal gene transfer. Plasmids may encode phenotypic modules which enhance overall bacterial fitness.
In this study we present the analysis of plasmid pP32BP2 of psychrophilic bacterium *Psychrobacter* sp. DAB_AL32B isolated from the ornithogenic Arctic soil.
Objectives: We aimed to verify the hypothesis that plasmid pP32BP2 is involved in the host adaptation to extreme Arctic environment.
Materials & methods: We used standard molecular biology methods (including: DNA sequencing, gene cloning, PCR, physiological tests) and common *in silico* methods for plasmid annotation (similarity searches, multiple sequence alignments, identification of protein conserved domains).
Results: The structural and functional examination of the plasmid pP32BP2 revealed that it carries three phenotypic modules which seem to be responsible for host adaptation to the Arctic habitat. Modules are potentially involved in: (i) type 3 fimbriae synthesis and biofilm formation (MRK module), (ii) osmo- and cryoprotection (BCC module) and (iii) carnitine catabolism (BCC and CAI modules).
Analyses of the MRK module revealed that this gene cluster significantly increases the host ability to adhere and, as a result, form biofilms. Examination of BCC and CAI modules revealed that they encode enzymes which catalyse consecutive steps of carnitine metabolism pathways. This suggest that carnitine may be utilized as the source of carbon and nitrogen. BCC module is also involved in glycine betaine and choline transport and metabolism, and therefore may have a crucial role in protection against high osmolarity and low temperatures.
Conclusion: Our study provides a valuable insight into the biology of psychrophilic bacterium *Psychrobacter* sp. DAB_AL32B. We showed that its plasmid pP32BP2 may play an important role in adaptation to extreme environmental conditions.

P HGT 3

Ecology and diversity of Antarctic psychrophilic bacteria isolated from ornitogenic soil

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Introduction: Antarctica is the coldest and most extreme region on Earth. However, even there some organisms (mostly bacteria and fungi) can survive. Those microorganisms are called psychrophiles and are perfectly adapted to living in this extremely cold region. Some of their beneficial, adaptive features psychrophilic bacteria acquired with plasmids.
Objectives: The aim of the study was to investigate the diversity of bacteria inhabiting soil from the penguins breeding colony in Antarctica, as well as their physiological properties. Moreover, we analysed the plasmids of isolated strains and their role in host adaptation.
Materials and methods: We used common microbiological and molecular methods to identify bacteria, analyse their physiology and isolate their plasmids. *In silico* methods were used for plasmid analysis.
Results: We have obtained 68 strains isolated from soil samples from penguin breeding colony located near the Arctowski Polish Antarctic Station. All bacterial strains were assigned to five genera, of which the most numerous was *Psychrobacter* (73,5%).
Physiological analyses showed that the majority of the strains have pH optimum above 7 and some of them are alkaliphiles. Moreover, some *Psychrobacter* strains tolerated very high salinity of medium, of even 10%. Heavy metal resistance analysis revealed that the presence of several multi-resistant strains, which was correlated with the heavy metals occurrence in this environment.
The plasmidome analysis revealed that the majority of analysed strains carry at least one plasmid. Moreover, about 15% is multi-replicon strains. Detailed bioinformatic analysis of those plasmids was performed.
Conclusion: The diversity analysis revealed the presence of bacteria belonging to five genera, which are well adopted to harsh Antarctic environment. The important role in their adaptation may play plasmids, and therefore, their analysis is essential for understanding the biology and diversity of psychrophilic bacteria.

P HGT 4

Organic fertilizers as a source of resistance genes and mobile genetic elements and their potential contribution to the spread of resistance genes in field soils

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Livestock manure is a reservoir of bacteria carrying antibiotic resistance genes (ARGs) that are often linked to mobile genetic elements (MGEs) such as transferable plasmids or integrons. Manure used as fertilizer might thus foster ARG spread among bacteria associated with the agro-ecosystem. Capturing of IncP-1ε plasmids by means of exogenous isolation from digestates of biogas plants (BGPs) using manure as co-substrate indicated that digestates might also contribute to the spread of ARGs and MGEs.
Our study aimed to elucidate whether ARGs and MGEs typically associated with manure are also present in digestates, and whether digestates spread in field soil enhance ARGs and MGEs similar to manures.
Sequences specific for IncN, IncP-1, IncW, IncQ and LowGC plasmids, class 1 and 2 integrons, disinfectant resistant genes (*qacE*/*qacE*Δ*1*), and ARGs (*sul1*/*2*/*3*, *tet*(A)/(M)/(X)) were detected in total community (TC-) DNA from manures of pig breeding and fattening farms and digestates of BGPs using manure by PCR/Southern blot hybridization. A field experiment was performed in order to investigate the effects of manure or digestate vs. inorganic fertilizer on the abundance of resistance genes and MGEs in bulk and rhizosphere soil. By quantitative real-time PCR ARGs (*sul1*/*2, tet*(A)/(M)/(W)/(Q)), *qacE*Δ*1*, class 1 and 2 integrons, and IncP-1, IncP-1ε and LowGC plasmids were quantified in TC-DNA of soil samples taken before and after fertilization, and immediately before harvest.
Class 1 and 2 integrons, IncQ and IncW plasmids, and most resistance genes monitored (except for *sul3* and *qacE*), were detected in all organic fertilizers. IncP-1 plasmids were found in all manures and most digestates, while IncN plasmids were exclusively detected in manures from breeding farms, and LowGC plasmids were only found in digestates and in one breeding farm manure. The spread of manure onto field soil temporarily increased the relative abundances of all resistance genes and integrase genes significantly in soils, while digestate application led to less pronounced or no effects. Plasmid specific sequences were below the detection limit in most samples of the field plot study.
Our data indicated that digestates contributed to a lesser extent to the spread of ARGs in soils than manures.

P HGT 5

Soil type-dependent effects of streptomycin and doxycycline applied with manure on the structure and resistome of soil bacterial communities

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Veterinary antibiotics, antibiotic resistant bacteria and resistance determinants located on mobile genetic elements are spread to agricultural soil with manure. For the evaluation of possible risks to human health, it is crucial to elucidate potential effects on the abundance and dissemination of antibiotic resistance in soil. However, not much is known so far about threshold concentrations of antibiotics in manure affecting the structure and resistome of soil bacterial communities.
In a microcosm study, a sandy and a loamy soil were mixed with manure and five concentrations of streptomycin or doxycycline (four replicates). Soil was sampled immediately after mixing as well as on days 28 and 92. Total community DNA was extracted and quantitative real-time PCR was used to assess effects of antibiotics in manure on the abundance of antibiotic resistance genes (*aadA*, *strA*, *tet*(A), *tet*(M), *tet*(W), *tet*(Q), *sul1)*, class 1 integrons (*intI1*) and associated quaternary ammonium compound resistance genes (*qacE+qacEΔ1*) as well as IncP-1 plasmids (*korB*) relative to 16S rRNA genes. Denaturing gradient gel electrophoresis of 16S rRNA gene fragments was used to evaluate effects on the bacterial community structure. Bi- and triparental matings will be used to capture and characterize mobile genetic elements potentially involved in the spread of antibiotic resistance determinants.
First results demonstrate a stronger effect of manure and antibiotics on the bacterial community structure and its resistome in the sandy soil, which was still detectable after 92 days. On day 28, positive correlations between streptomycin concentration and the relative abundance of *aadA* and *qacE+qacEΔ1* were observed in the sandy soil only. Consistently, the concentration of doxycycline was positively correlated with the relative abundance of tetracycline resistance genes *tet*(A), *tet*(M), *tet*(W), *tet*(Q) as well as with *intI1*, *aadA*, *sul1*, and *qacE+qacEΔ1* in the sandy but not in the loamy soil.

The establishment of threshold concentrations for antibiotics in manure remains challenging. However, the results indicate a higher susceptibility of sandy soil bacterial communities, and these soil type-dependent effects should be considered in future risk assessments.

P HGT 6

Analysis of Horizontal gene transfer systems in members of *Roseobacter* lineage.

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The *Roseobacter* lineage is a diverse and phylogenetically coherent group within *α*-proteobacteria class that can represent up to 20% of marine bacterioplankton. Their genetic repertoire allows them to colonize a variety of habitats. In spite of this genetic diversity, previous studies based on sequenced genomes showed that this group has a *core* genome transmitted vertically that allow to outline a robust phylogeny in five different clades.
Our aim was to study the relevance of three different mechanisms of horizontal gene transfer (HGT): gene transfer agents (GTAs), *rep*ABC plasmids, and insertion sequences (IS) in genomes of *Roseobacter* lineage sequenced up to date, as a way of approaching *Roseobacter* genetic diversity.
All genic products were identified by sequence homology using blastp. Phylogenies were done using parsimony and a bootstrap of 100. IS were grouped according to sequence identity and assigned to families with IS finder.
GTAs are phage-like elements of genetic exchange. Its genetic cluster consists in 15-17 genes, 12 of which maintain their synteny in 88% of analyzed genomes. The phylogenetic analysis of the concatenate of 12 genic products agreed with the phylogeny of the *core* proteome. These results show that this genetic structure has a high level of conservation and, therefore, GTAs would be considered an ancestral HGT system among members of *Roseobacter* lineage.
*Rep*ABC plasmids are widely distributed among *α*-proteobacteria, and were found in some of the analyzed genomes. We established a phylogeny based on RepC replicase. In this case, the topology of the tree disagreed with the phylogeny of the *core* proteome. This result suggests that *Rep*ABC plasmids are transferred promiscuously among roseobacters.
Finally, transposases were detected in all genomes. Their analysis revealed the presence of, at least, members of 28 different IS families. We analyzed the transposases shared among genomes from the phylogenetic clades and among the genomes within each clade. This approach is allowing us to propose possible flows of ISs among roseobacters.
In conclusion, we have demonstrated the presence of three different and active mechanisms of HGT that might have contributed to genetic diversity and plasticity in nearly all the *Roseobacter* lineage members sequenced up to date.

P HGT 7

Dissemination of ESBL genes within microbial communities in river sediments

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Enterobacteriaceae isolates sampled from upstream and downstream of a wastewater treatment plant (WWTP) in Finham were investigated with respect to plasmid borne antimicrobial resistance gene presence. Only isolates possessing resistance to 3rd generation cephalosporins (3GCs) were investigated further. Resistance to other antibiotics (including fluoroquinolones and aminoglycosides), biocides (specifically quaternary ammonium compounds (QACs)) and heavy metals was further investigated. The transferability of these plasmids was also investigated to determine promiscuity of these large conjugative plasmids under different selection pressures (including QACs and heavy metal selection) and to determine whether transfer of antibiotic resistance genes (ARGs) could be selected for indirectly. We hypothesised that ARG could be co-selected for by environmentally induced selection pressures propagating the environmental antibiotic resistome. Results suggested ARG could be maintained via QAC selection pressures, and that the F-type plasmids were the most likely to transfer under this pressure. We found the most likely ARGs to transfer were $bla_{ctx\text{-}m}$ and *aac 6'* conferring resistance to 3GC and aminoglycoside and fluoroquinolones respectively.
We also aimed to investigate the host range of these F-type plasmids with the hypothesis that plasmid transfer is likely to occur from *E. coli* to other environmental bacteria under 3GC and QAC resistance selection. We conducted further biparental matings experiments with the same *Escherichia coli* isolates from Finham (with multidrug resistance containing plasmids) and environmental Enterobacteriace isolates (including *Klebsiella, Aeromonas* and *Citrobacter*) as recipients.

Adaption and the role of HGT

To determine any fitness costs associated with possession of these large conjugative plasmids, growth curve experiments were carried out. Preliminary results indicate plasmid possession has a significant fitness cost with an increased doubling time of approximately 1.5 times that of wildtype *E. coli.* Experiments testing plasmid stability and maintenance under a range of conditions were also conducted.
In conclusion these preliminary results indicate ARGs can be transferred via indirect selection and consequently the use, and release of biocides in to the environment may increase the environmental resistome.

P HGT 8

Transposases and Insertion Sequences in *Ferroplasma acidarmanus* fer 1 shared homology with fifteen *Bacteria and Archaea*

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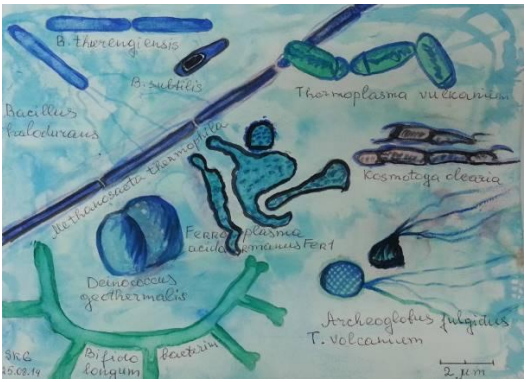
Objective: Produce comprehensive summary on transposable elements belonging to the extremophile, *Ferroplasma acidarmanus Fer1*, using comparative genomic analysis.
Design & Methods: Genes from *F. acidarmanus* that were associated with transposase activity were derived from the IMG-ACT database. The functions of the genes were analyzed using sequence similarity tools linked through IMG-ACT, IS Finder and extensive literature searches. Using IMG-ACT, 71 putative genes were identified to code for transposases in *F. acidarmanus*. These genes were further analyzed using the protein BLAST for the non-redundant (nr) protein sequences, Swiss-Prot, and ISfinder databases. Maximum likelihood models were used within Phylogeny.fr and Mega 6 for phylogenetic analysis. The genes were quantitatively affiliated with evolutionary ancestors and transposon families.
Results: Summary of our results using both IS finder and IMG-ACT on transposases and IS element in both *F. acidarmanus* and the orthologous hosts is presented. We identified 15 ortholog hosts which provided *F. acidarmanus* with genes coding for transposases and integrases. Remarkably, *F. acidarmanus* shared these genes with both *Archaea* and *Bacteria* which were capable of survival at low pH or high temperature such as *Lactococcus lactis, Sulfolobus solfataricus, Archaeoglobus fulgidus, Thermoplasma acidophilum, T. volcanium* and *Bifidobacterium longum* as well as the spore producing Bacillus species including *B. thuringiensis*, *B. halodurans*, *B. cereus*, *B. subtilis*. Phylogenetic analysis demonstrated evolution of the studied IS families. New model of the horizontal gene transfer of the IS families and transposases dominating in *F. acidarmanus* was produced. Based on the analysis of 71 transposases we identified ~12 families of IS elements in *F. acidarmanus*, which indicated incredible potential for the mutagenesis and horizontal gene transfer. Most of the transposases shared between *T. volcanium* and *F. acidarmanus* belonged to **IS200 and ISNCY** families. We identified earlier unknown **IS607** and **IS982** families of IS element in *F. acidarmanus*.
Conclusions: Seventy-one IS-elements found in *F. acidarmanus could have* provided the organism with potential for the genetic diversification of the population and selection of variants better fitted for the extreme environment

Figure 1



P HGT 9

Genome sequences of thermophilic sulfur disproportinating beterium *Thermosulfurimonas dismutans*.

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Recently described *Thermosulfurimonas dismutans* is a thermophilic, anaerobic, chemolitotrophic bacterium. This microorgansism was selected for genome sequencing, because it is the first genomes of thermophilic sulfur-disproportionating bacterium. *T. dismutans* grew anaerobically with elemental sulfur as an energy source and CO2 as a carbon source and elemental sulfur was disproportionated to sulfide and sulfate. Crystalline iron(III) oxide is used as sulfide-scavenging agent by *T. dismutans.* A total of 2164 open reading frames were predicted from its 2.1 Mbp genome. Analysis of the sequenced genome *T. dismutans* revealed clusters of genes which products are involved in sulfur metabolism. The genes of dissimilatory sulfate reduction pathway including adenylylsulfate reductase, sulfate adenylyltransferase, dissimilatory sulfite reductase were found. Also genes encoding nitrogen-fixation and carbon-fixation pathways were identified. The genome contains all the genes that are required for flagellum formation.

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P HGT 10

Evaluating the differences in inactivation kinetics upon solar irradiation of *Escherichia coli* with and without New Delhi metallo beta-lactamase gene

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Emerging biological contaminants in raw and treated wastewater have been the subject of much attention and growing recognition in recent years. These merging contaminants include antibiotic-resistant microorganisms and the genes conferring this resistance. Upon release into the environment, these resistant microorganisms can compromise public health. The risk is further aggravated by the possibility of horizontal gene transfer events that can lead to the creation of superbugs and/or antibiotic resistant pathogens and opportunistic pathogens. Little is know about the fate of these contaminants.

Our study sets out to collect information on this by examining the response of two bacterial isolates under stressing environmental conditions. The first isolate is *Escherichia coli* strain PI-7 that was isolated from local wastewater influent, and was determined to possess a plasmid that was horizontally acquired from *Klebsiella oxytoca*. Sequencing of the plasmid further revealed the presence of bla_{NDM}, bla_{TEM}, aadA, armA, rmtC, msrE and mphE. The second isolate is *E. coli* DSM1103 that has been tested to be susceptible to a wide spectrum of antibiotics. Isolates were selected to represent pathogenic and highly drug-resistant wastewater bacteria and non-resistant bacterial counterparts.

Bacterial suspensions of pure cultures were placed under simulated solar irradiation over a number of hours, and the inactivation of the bacteria isolates were monitored by periodic sampling and plate counting. Our results showed solar irradiation achieving 5-7-logs inactivation of a *E. coli* DSM1103 over 6 h of exposure. In contrast, *E. coli* PI-7only showed 3-4.5-logs inactivation over an extended 12 h of exposure. These findingsimply that drug-resistant *E. coli* PI-7 may have acquired plasmids or encode for certain genetic elements that may confer advantages in stressing or extreme conditions leading to prolonged survival.

Next steps in the study include monitoring the changes in the transcriptome over the trial period to assess differential gene expression, and to also monitor plasmid retention and loss.

P HGT 11

Integron diversity in bacterial communities of freshwater sediments is impacted by contamination

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Integrons are genetic elements known to be involved in the adaptation and the evolution of pathogenic bacteria. Although they have been discovered into clinical contexts, they are ancient structures found into other environments. But their role in bacterial communities of natural environments is still in question. Their structure is composed of the *intI* gene, encoding an

integrase, IntI, and a promoter allowing the expression of a succession of gene cassettes. These gene cassettes are mobilisable thanks to the activity of the integrase. Thus, integrons are both genes reservoirs and expression systems that are able to acquire and spread genes conferring selective advantages face to a selection pressure. Although several surveys have focused on gene cassette diversity into bacterial communities under different levels of contamination outside clinical environments, the diversity of integrase has been poorly investigated, especially into freshwater environments. Indeed, to date the clinical integrons have been mainly followed.
The work presented here aimed to describe the integrase diversity in bacterial communities from freshwater sediments along the *Gave de Pau* River (France) at sampling stations with different contamination levels and contaminant types.
From the metagenome of each station, IntI libraries were constructed. The gene cassette pool was also characterized regarding the gene cassette size. The IntI and gene cassette data were analysed by nMDS and compared with the bacterial community structure assessed by 16S rRNA gene T-RFLP.
322 novel IntI sequences were identified revealing a wide diversity, larger than that previously expected in non-clinical environments. The bacterial community structures did not fully explain the integron diversity. The integrase diversity tends to be link to the contamination level while the gene cassette diversity related to the contaminant type.
These results provide further elements that argue the non-clinical integron would be involved in the adaptation of bacterial communities in response to the presence of contaminants in natural environments.

P HGT 12
Insect symbionts and plant pathogens share
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Horizontal Gene Transfer (HGT), one of the major evolutionary drivers in bacteria, facilitates novel acquisition of ecologically important functions in changing environment. Plant pathogenic bacteria, transmitted by various insects, necessarily adapt to two very different habitats and their associated microbial communities, i.e. plant sap and insect host. Here, we investigate genomic data available for *Liberibacter* species, economically important plant pathogens, and report number of transfers from facultative symbionts of the *Liberibacter* psyllid vectors, e.g. *Sodalis*, *Arsenophonus* and other related bacteria. The extent of the HGT in some *Liberibacter* species is substantial and includes core gene clusters expanding metabolic and transfer capacity of the bacteria with e.g. tryptophan biosynthesis or iron acquisition through ABC transporters. We highlight a possible evolutionary importance of HGT between two distinct ecological types of bacteria: plant pathogens and insect symbionts, and point out the importance of insect host microbiome as a functional pool for HGT.

P HGT 13
Viral-host interactions in the environment and their adaptive significance
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Interactions between the members of a microbial community can lead to their adaptation to the environment. Among the many interactions that take place in an ecosystem, the one involving phages and their hosts has been seen to play a major role in microbial diversity and population dynamics. While viruses and phages can mediate the transfer of genetic material between microorganisms (transduction), which could be a mechanism for rapid adaptation, the relationship between them and their hosts in natural environments is poorly understood. Thus, measuring the impact that transduction could have on microbial communities is a difficult task.
Here, we propose a workflow that uses CRISPRs to describe viral-host interactions in the environment. By retaining viral sequences from past infections, clustered interspaced short palindromic repeats (CRISPRs) could help us link viruses and phages with their microbial hosts. Infection networks are created where viruses are connected to the microbial cells they infect, using metagenomic data. Furthermore, we can search for transduction events in metagenomic data by looking for viral sequences containing microbial DNA. If we can track the source of the viral sequence, we can then use our infection network to see which microbial cells this virus might have infected. Thus, we can learn about the distribution of transduction events in a microbial community.

Recently, viruses in the cryosphere have been seen to be abundant, highly active and with broad host ranges, characteristics that could make viral transduction a key driver of adaptation in these environments. The workflow described above was used to analyse viral-host interactions in habitats subject to sub-zero temperatures.

P HGT 14

The bovine plasmidome, a genetic hub for microbial genetic communication

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Plasmids are self-replicating genetic elements capable of mobilization between different hosts. They often serve as mediators of lateral gene transfer, a process considered as a sculpting evolutionary force in microbial environments. Our aim was to characterize the overall plasmid population the bovine rumen niche, which houses a complex and dense microbiota that holds enormous significance for humans. We developed a procedure for the isolation of total rumen plasmid DNA, termed rumen plasmidome, and subjected it to deep-sequencing and analysis using public and custom-made bioinformatics tools. A large number of plasmidome contigs aligned with plasmids of rumen bacteria isolated from different locations and time points, suggesting that not only the bacterial taxa, but also their plasmids, are defined by the ecological niche. Whereas most studies examining the identity and extent of laterally transferred genes focus on identifying the tracks of such events in bacterial genomes, this study presents the opportunity to examine the identity of such genes and the phylogenetic barriers they cross on the transfer "vehicles." Evidently, the rumen plasmidome is of a highly mosaic nature which can cross phyla. Interestingly, when we compared the functional profile of the rumen plasmidome to two plasmid databases and two recently published rumen metagenomes, it became apparent that the rumen plasmidome codes for functions which are enriched in the rumen ecological niche and could confer advantages to their hosts, suggesting that the functional profiles of mobile genetic elements are associated with their environment, as has been previously implied for viruses.

P HGT 15

Identifying large conjugative plasmids in draft genomes

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Draft genome sequences of microbial strains are becoming more abundant, with the advent of cheap sequencing and more approachable bioinformatics. In fact, environmentally important bacteria are now often sequenced for the purpose of identifying one or a few phenotype-defining genes, such as a catabolic pathway for a given compound. These studies are often concluded by identifying the gene, but not on which DNA molecule the genes are situated. It is of ecological importance whether these genes are a conserved part of the chromosome or if they are located on conjugative plasmids that can be rapidly dispersed through the microbial community. Determining what contigs in a draft genome assembly that belongs to a conjugative plasmid and which are chromosomal is not a trivial task, since the large plasmids are often break into several contigs in the assembly. Recently, a tool was developed that can predict small circular plasmids in metagenomes[1], which successfully identified hundreds of plasmids in the size range 1,000 - 13,000 nt. The aim of this project is to develop an *in silico* tool that can identify contigs constituting larger conjugative plasmids, based on the presence of plasmid marker genes and genetic context. The contig scaffold information from genome assemblers such as SPAdes [2] is used to extract contigs associated with the contig(s) identified as a putative conjugative plasmid. Finally, a novel method of classifying plasmids is attempted, based on their total genetic content of plasmid-related genes, rather than the classical approach of looking at single plasmid incompatibility determinants, such as the replication initiation proteins (Rep). The tool is tested on simulated paired-end Illumina MiSeq sequencing of a large number of complete genomes with known plasmids from different microbial families.

1. Jorgensen TS, Xu Z, Hansen MA, Sorensen SJ, Hansen LH (2014) Hundreds of circular novel plasmids and DNA elements identified in a rat cecum metamobilome. PLoS One 9: e87924.
2. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, et al. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19: 455-477.

P HGT 17

Comparative metamobilomics of rat-gut bacteria from pristine islands, rural areas and hospital sewers

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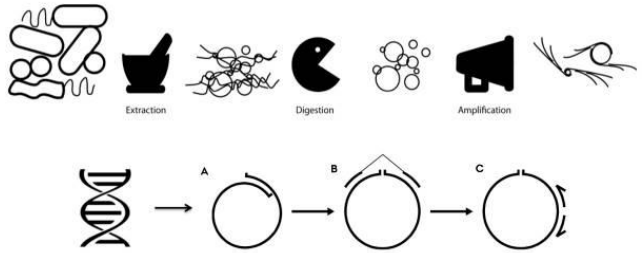
Plasmids are considered the main vehicles between bacterial genera in evolution and dissemination of antibiotic resistance, virulence factors or xenobiotic degradation pathways. Contrary to popular belief, the majority of plasmids in natural environments carry no accessory genes. Earlier methods of investigation have favored the discovery of plasmids containing selected traits. Recently, several metagenomic approaches have evolved to investigate the naturally non-selected gene pool of environmental microbes. We have earlier demonstrated that one rat gut mobilome contain hundreds of hitherto unknown circular plasmids and DNA elements with few accessory genes. In this study we present the mobile gene pool in rat gut microbiota from environments of contrasting anthropogenic pressures to investigate the environmental effects on the plasmid presence and diversity. Brown rats from Danish hospital sewers and urban settings, and brown rats from remote and pristine islands in the Falkland Islands archipelago were sampled and chromosomal-DNA-free mobilomes were produced from cecal content and subsequently sequenced on the illumina HiSeq platform. Identification of complete, circular plasmids within the assembled sequences was performed with a tailor-made pipeline verifying circularity independently twice. Using this methodology, we compare hundreds of newly discovered, completely assembled, circular and novel plasmids between the geographically separated and contrasting environments. Further, metagenomic analysis on antibiotic resistance genes and virulence genes and 16S rRNA genes from the rat bacteria is compared to describe the environmental influence on the rat gut microbiota.

Figure 1



P HGT 18

ComQXPA quorum sensing systems may not be unique to *Bacillus subtilis*: a census in prokaryotic genomes

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The comQXPA locus of *Bacillus subtilis* encodes a quorum sensing (QS) system typical of Gram positive bacteria. It encodes four proteins, the ComQ isoprenyl transferase, the ComX pre-peptide signal, the ComP histidine kinase, and the ComA response regulator. These are encoded by four adjacent genes all situated on the same chromosome strand. Here we present results of a comprehensive census of *comQXPA*-like gene arrangements in 2620 complete and 6970 draft prokaryotic genomes (sequenced by the end of 2013). After manually checking the data for false-positive and false-negative hits, we found 39 novel com-like predictions. The census data show that in addition to *B. subtilis* and close relatives, 20 *comQXPA*-like loci are predicted to occur outside the *B. subtilis* clade. These include some species of Clostridiales order, but none outside the phylum Firmicutes. Characteristic gene-overlap patterns were observed in comQXPA loci, which were different for the *B. subtilis*-like and non-*B. subtilis*-like clades. Pronounced sequence variability associated with the ComX

peptide in B. subtilis clade is evident also in the non-*B. subtilis* clade suggesting grossly similar evolutionary constraints in the underlying quorum sensing systems.

P HGT 19

Horizontal gene transfer through plasmid transport: Heavy metal and antibiotic tolerance in bacteria

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Question: Urban soil pollution is a common problem, of which heavy metals are one of the most significant pollutants. Heavy metal tolerance in bacteria is observed when samples are collected from urban soil, due to high concentration of heavy metals in the soil. Correlation has been observed between heavy metal tolerance and antibiotic tolerance in bacteria, indicating occurrence of tolerance against number of heavy metals and antibiotics in a single bacterial strain. Multiple stress tolerance in bacteria is a serious threat to the mankind, especially when revealed in pathogenic bacteria. The most common transmissible instrument for resistance among bacteria is the R-plasmid.
Methods: The aim of the present study is to monitor horizontal gene transfer of plasmid-determined stress tolerance under lab conditions. E. cloacae (DGE50) & E. coli (DGE57) were used throughout the study. Samples were collected from contaminated soil to isolate bacterial strains having tolerance against heavy metals and antibiotics.
Results: We have demonstrated plasmid transfer, from Amp+Cu+Zn- strain (DGE50) to Amp-Cu-Zn+ strain (DGE57), producing Amp+Cu+Zn+ transconjugants (DGETC50→57) and Amp+Cu-Zn+ transformants (DGE TF50→57). DGE57 did not carry any plasmid, therefore, it can be speculated that zinc tolerance gene in DGE57 is located on chromosome. DGE50 was found to carry three plasmids, out of which two were transferred through conjugation into DGE57, and only one was transferred through transformation. Plasmid transferred through transformation was one out of the two transferred through conjugation. Though the results of transformation it was revealed that the genes of copper and ampicillin tolerance in DGE50 are located on separate plasmids, since only ampicillin tolerance genes were transferred through transformation as a result of one plasmid transfer.
Conclusion: By showing transfer of plasmids under lab conditions and monitoring retention of respective phenotype via conjugation and transformation, it is very well demonstrated how multiple stress tolerant strains are generated in nature.

P HGT 20

Bacterial community shifts and horizontal gene transfer assessment in silver stressed soils

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Silver (Ag) selective pressure on bacterial communities is of interest due to the use of Ag nanotechnology in medical and commercial products, and the subsequent release of Ag via the wastewater-biosolids-soil pathway. Using partial 16S rRNA gene Illumina sequencing and X-ray absorption near edge spectroscopy (XANES) to monitor changes in Ag^+ stressed soils, we have previously observed: rapid transformation of Ag^+ into less labile forms; changing bacterial diversity with time; and the selection of metal/halo-tolerant strains and opportunistic pathogens.
We have since explored silver-induced microbial selection across a broader range of soils and exposure scenarios, and assessed the transfer of known Ag and tetracycline resistance genes under both realistic soil Ag concentrations and highly selective conditions. In this study, 9 soils were treated with concentrations ranging from 1 to 2,000 mg Ag^+ kg^{-1}, leached with artificial rainwater, and incubated for 2.5 months. Soil characterisation included: particle size distribution, pH, electric conductivity, total carbon (TC) and nitrogen (TN), effective cation exchange capacity, and 16 major/trace elements. Changes in Ag lability and form were determined using advanced chemical techniques (DGT, XANES), and microbial respiration and the activities of 8 exo-enzymes were assessed using high-throughput microplate assays. Total DNA extracts were used to determine 16S rRNA gene diversity and quantity, and *tetG* and *silE* marker genes linked to tetracycline and silver resistance are also being quantified.
Weibull and log-logistic modeling provided robust fits for microbial respiration rate responses in this highly diverse soil collection. Nominal EC_{50} values for respiration ranged from 3.9-316.8 mg kg^{-1}. Enzyme activity responses were quite diverse, covering a similar range of EC_{50} values. Some provided robust fits for hormesis models.

These results show that realistic soil Ag concentrations (e.g. in biosolids-amended soils) are sufficient to impact on microbial respiration activity, but the response is soil dependent. Molecular results revealing links between actively participating microbial groups and enzyme activities; and assessing the potential for horizontal resistance gene transfer under conditions of Ag stress will also be presented.

P HGT 21

Conjugative DNA Transfer in *E. coli* Isolates Increases Antibiotic Resistance in Urban Waterways

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The city of Milwaukee urban waterways represent a natural reservoir of antibiotic resistance, which may provide a source of transferable genetic elements to human commensal bacteria and pathogens. We hypothesize there is a greater abundance of multidrug-resistant bacteria and transferable genetic elements in the urban waterways compared to the resistance of the human microbiome of the city. The phenotypic antibiotic resistance of 259 *Escherichia coli* isolated from the urban waterways of Milwaukee, WI compared to those from sewage and a clinical setting in Milwaukee was determined based on antibiotics covering 10 different families. All obtained isolates were determined to be multi-drug resistant. *E. coli* from urban waterways demonstrated a greater incidence of resistance to higher numbers of antibiotics compared to the human derived isolates. Of the 219 strains identified with plasmids, a total of 47 distinct agarose gel plasmid banding patterns were identified. Six of those were more abundant and they conferred various patterns of antibiotic resistance in their hosts. Plasmids with patterns P1 and P2 were most abundant in *E. coli* isolated from the effluent sediment compared to other locations. Host bearing the P1 and P2 patterns were associated with resistance to above 4 and up to 12 different antibiotics. Some of the plasmids are directly transferable between bacteria through conjugation or transformation. The presence of class 1 and class 2 integrons determined by PCR amplification of integrase gene (*intI*) was greatest in the clinical *E. coli*. Molecular typing and PCA and Chi-square comparison indicate that the presence of plasmids in the environmental isolates correlate with the resistance to a greater number of antibiotics or location, but no correlation was determined with the presence of integrons. Milwaukee's urban waterways select for a greater incidence of multi-drug resistant bacteria and also harbor an extensive mobile resistome. Plasmid sequencing and ongoing data mining will reveal detailed information about this mobile resistome that will be presented. The implications of this study are significant to understanding the presence of resistance in urban freshwater environments by supporting the idea that sediment from urban waterways serves as a reservoir of antibiotic resistance.

P HGT 22

Urban wastewater treatment plants and dispersion of carbapenem resistant bacteria into the environment

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Introduction: Urban wastewater treatment plants (UWTP) represent a key dissemination pathway for bacteria and resistance traits to and from humans; however, AR studies frequently focus on indicator or human pathogens microorganisms in wastewater (e.g., thermotolerant coliform), even though they cannot compete with environment bacteria, reducing their presence in the water. This project used highly selective, culture-dependent approaches (chromogenic agars) coupled to MALDI-TOF mass spectrometry to determine the prevalence and identity of carbapenem resistant bacteria (CRB) in an UWTP and how it affects the dispersion of ARGs to the environment.
Material and Methods: This study specifically monitored an UWTP in Northern Spain, which included primary and secondary treatment (conventional activated sludge), and river waterand sedimentabove and below the UWTP in September 2014. Influent, effluent and intermediate locations in the UWTP and also sediment and water column samples were collected and analysed using different culturing media, including: MacConkey agar to quantify total (37 ºC) and thermotolerant coliforms (44 ºC), and chromogenic agars designed to detect extended-spectrum β-lactamase (chromID ESBL, bioMérieux) and carbapenem (chromID CARBA, (bioMérieux) resistant gram-negative bacteria (total and thermotolerant). Matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) was used to identify specific resistant bacterial isolates.

Results and Conclusions: This study show that carbapenenem resistant bacteria from UWTP and river sources differ from clinical samples, being dominated by *Pseudomonas spp., Aeromonas spp.., and Acinetobacter spp.* (*Enterobacteriaceae* close-related bacteria). Furthermore, unit operations in the UWTP efficiently reduces the total number of bacteria as well as resistant bacteria, especially total coliforms, in final effluents, but showed bacterial increases in the waste sludge. However, thermotolerant bacteria displayed a different pattern, decreasing both through the WTP and also sludge samples. Additionally, although the UWTP seemed to be reducing ARB in the treatment processes, very high levels of ARB were found downstream UWTP. Further examination showed that this was likely because the wastewater inputs to the WTP were in combined sewers, which were overloaded during storms and transiently releasing untreated sewage directly to the river.

Oral presentations

O CMC 1

Noise and competition: bacteriocin role in Escherichia coli populations

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In the never ending battles for resources and space bacteria use every mean at their disposal. But by far the most popular weapon used by bacteria are target specific antibiotics, the bacteriocins. These are proteinaceous antibiotics produced against close relatives to the producing cell, competing with it for the same resources, and mediating bacterial interactions. In *Escherichia coli* populations interactions are often mediated by bacteriocins named colicins that have been studied for almost a century and their ecological role, evolution, structure and function are well documented. However, the vast majority of studies examined planktonic cultures though in their natural environment, i.e., the gastrointestinal tract, *E. coli* form biofilms. We predicted that increase in biofilm environments would augment colicin expression similar to other species that express bacteriocins solely in biofilms. We further speculated that colicin expression would result in stochastic phenotypic heterogeneity in the colicinogenic mono-population. To test our hypotheses we examined colicin expression in planktonic and biofilm environments at the single cell level. We also imposed aerobic and anaerobic conditions, as cells residing in the gastrointestinal tract experience both, and monitored colicin expression under these conditions.
Five-folds increase in colicin expression was monitored in biofilm compared to planktonic cells. Upon examining the colicinogenic biofilms at a single cell level we observed two distinct subpopulations: small groups of cells forming hot-spots of colicins, randomly distributed in the matrix, while the majority of the population silenced colicin expression. We further tested colicin expression in biofilms cultivated with or without oxygen and found that they did not differ in their colicins expression. This may suggest that as portions of any biofilm is anaerobic or oxygen limited, colicins did not evolve to respond to shifts in oxygen concentrations.
We conclude that bacterial defense systems are differentially expressed in a structured population to avert invaders while avoiding the high cost associated with expression. Yet this defense mechanism is mediated by phenotypic noise evolved to balance security and cost to the population responding to structure and culture conditions.

O CMC 2

Testing neutral and niche processes which shape microbial communities

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Two main theories have been discussed to explain the processes that structure ecological communities: The classic niche theory asserts that stable coexistence requires ecological differences among species for promoting diversity whilst the neutral theory assumes that the prospects of individual organisms are independent of species identity, with stochastic processes playing a key role in shaping biodiversity. However, testing the mechanistic processes of species coexistence and community assemblage is limited by the demands for long-term temporal data sets, which are thus rarely collected in plant/animal communities. Such time-series can be easily obtained with microbial communities. In our study, we constructed spatially implicit microbial communities that contain four competing bacterial species in presence or absence of a protist predator. These assembled communities were maintained under a chemostat regime and were sampled daily to track dynamics of each single species and to follow a selected microbial function with and without interactions across trophic levels. By using econometric models and methods, we show that both equalizing (e.g. stochastic mortality) and stabilizing (e.g. anti-predation strategies) processes may operate simultaneously on the communities. Since both ecological and economic systems are complex systems comprised of autonomous agents and individuals, econometric techniques seem to be promising to infer the causalities of these mechanistic processes. Our study will thus add significant theoretical and methodological insights to mechanistically understand microbial community dynamics.

O CMC 3

High-wire acts: Mycelia as hot spots for horizontal gene transfer of bacteria

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Introduction: Horizontal gene transfer (HGT) enables soil bacteria to adapt to changing environments by acquiring new genetic traits such as resistance to antibiotics and heavy metals or new degradative pathways. Conjugation is believed to be the most important mechanism of bacterial adaptation in soil, involving the transfer of plasmids via direct cell-to-cell contact. Yet, soil bacteria typically live in isolated surface-associated colonies. Hence, HGT between them requires cell movement in soil water or along surfaces, which however are typically discontinuous in nature.
Objectives: We studied the effects of bacterial dispersal along mycelial networks to investigate (I) if mycelial networks can help bacteria to overcome spatial isolation and (II) how these networks influence bacterial HGT.
Materials & methods: Using laboratory-scale microcosms, we applied a bacterial reporter system for HGT events consisting of two *Pseudomonas putida* strains, labelled with distinct fluorescent proteins, as plasmid donor and recipient. Resulting transconjugants were indicated by an emerging third fluorescence signal. Successful conjugation events along the hyphae were both quantified with flow cytometry and visualized by fluorescence microscopy. To obtain more generic, spatio-temporal information on the role of mycelial networks for HGT in heterogeneous habitats, an individual-based simulation model was employed.
Results: Our results show that (I) mycelial networks can help bacteria to overcome spatial isolation and (II) that mycelial structures promote bacterial HGT by providing a transport network and confined aqueous films in which bacterial contacts are more frequent, leading to a significant increase in transconjugant cells. Individual-based simulations supported these observations and revealed that the tendency of bacteria to concentrate around mycelial networks, resulting in high bacterial densities along hyphae, has a more pronounced effect on HGT than the promotion of bacterial dispersal by the mycelium.
Conclusion: Our study shows the beneficial role of the mycosphere on bacterial HGT by providing transport networks that facilitate cell-to-cell contact, leading to significantly increased gene transfer.

Poster presentations

P CMC 1

Continuous culturing as ideal model system to study the potential spread of antibiotic resistance in aquatic microbial communities

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Question: Our aim is to test the anthropic impact on the ecology and evolution of natural communities, namely to test the impact of low-dose antibiotics on the bacterial community composition, on its fitness, and on resistance and resilience of natural communities to invasion by potential pathogens.
Methods: The implementation of continuous culture systems (chemostats) is a well-known lab practice in microbial ecology. Chemostats of different complexity and size have been use to experimentally demonstrate a number of important theories in modern ecology. Coupling continuous culturing, molecular biology, and flow cytometry we developed a complex lab based system to mimic environmental conditions.
We performed a number of experiments with artificially constructed communities and with natural communities adapted to the chemostat.
Results: In a first experiment an artificial community composed by five different bacterial strains was exposed to a cocktail of three antibiotics in low and very low concentration. The impact of antibiotics resulted in a 75% reduction of bacterial abundances, without any significant modification on the community composition, independently by the concentration.
In a second experiment the natural microbial community from Lake Maggiore (IT-CH) was exposed in continuous cultures to tetracyclines in low and very low concentration, and later invaded by three *E.coli* strains isolated from the Venice Lagoon. The natural community composition was analyzed through NGS analysis, while potential presence of resistance genes was measured by RT-PCR, and bacterial and flagellates abundance by flow cytometry.

Conclusion: None of the strains in the first experiment had known resistant genes, but thanks to the interaction within strains, the community developed a new resistance strategy, through co-aggregation.
The second study is the first to test the impact of antibiotics on a natural bacterial community in continuous culture, in relation to the resistance to invasion by a potential pathogen. Our results highlight the risk posed by the anthropic modifications of interactions within natural bacterial communities.

P CMC 2

Understanding reactions of bacteria introduced into contaminated soil systems with the purpose of bioremediation

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Bioaugmentation, or the inoculation of specific pure or mixed cultures into contaminated sites with the purpose of increasing biodegradation rates, has attracted considerable interest but attained mixed results. One of the major reasons to the variable success of bioaugmentation is our lack of understanding of the reactions of the introduced bacteria into their "new" environment. We set out here to compare the behaviour of a number of different bacterial strains (i.e., Sphingomonas wittichii, Pseudomonas veronii and Arthrobacter chlorophenolicus) with useful degradation properties upon introduction into soil systems.
We use two different types of techniques and experiments to study the behaviour of inoculated strains. In the first type we study the changes in genome-wide gene expression of the introduced strains upon inoculation into sandy soil systems, or upon growth within the sandy soil, in comparison to growth in liquid culture. For a global behavioral comparison among different strains, we study genome-wide expression differences upon exposure to compounds inferring water stress. In all cases, expression differences are interpreted using Gene Ontology terminology. In the second type of experiment, we use gene reporter technology to measure and interpret the variation in catabolic pathway expression among individual cells within the sandy soil in the presence or absence of specific contamination.
Our results show massive gene expression differences upon inoculation and growth in sandy soil compared to liquid, indicating that cells completely rewire their physiology despite maintaining the same overall growth rate. Global comparison among strains indicates a variety of different strategies in response to water stress but little commonalities. Reporter gene measurements suggest substrate bioavailability and metabolite competition to be important for inoculant success.
Reference: Moreno-Forero, S. and J. R. van der Meer. ISME J. (2014) doi:10.1038/ismej.2014.101

P CMC 3

Microbial ecology approach of anodic biofilms for the study of scaled-up microbial fuel cells.

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Since 1911 when Potter described bacteria that could produce electrical currents interest in this ability and associated technologies like microbial fuel cells (MFCs) has grown especially the last 20 years. Many MFCs have an anaerobic chamber filled with the feeding solution where are immerged an anode and a cathode which is generally in contact with air for oxygen supply. After inoculation with bacteria (pure bacterial culture or from environmental sources such as wastewater), biofilms develop on the anode and cathode. Some bacterial strains are able to use the anode as their terminal electron acceptor. These electrons travel via an external electric circuit to the cathode where the oxygen reduction occurs with or without bacterial participation.
The application of MFCs for the production of energy from wastewater requires the scale-up of such devices which, for now, doesn't allow electrical yield comparable to lab-scale MFCs. Our work was based on the hypothesis that typical scale-up efforts modify specific MFC parameters (substrate diffusion, geometry, hydraulic forces…) which in turn affect the anodic biofilm (microorganism selection, physical structure of the biofilm, electrons transport strategies…) and that is one major reason for electric yield variations. We investigated microbial ecology and electricity production of three MFCs of different volumes: 10 mL, 500 mL, 4 L operated under similar conditions and identical MFC parameters.
Electrical production and microbial community structure (16S rRNA gene sequencing to evaluate the enrichment of electroactive bacteria species like *Geobacter sulfurreducens* or *Shewanella oneidensis MR-1*) and function (to examine food web and electron exchange functions) were monitored during the MFC start-up and operation. In parallel, the physical structure of the biofilms (such as density, thickness, bacterial distribution and nanowire (conductive pili) presence) was studied by microscopy techniques and proteomics analysis.

P CMC 4

Unravelling the relationship between indigenous community diversity and success of bioaugmentation using synthetic microbial ecosystems

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Introduction: According to EU guidelines, pesticide residues like 2,6-dichlorobenzamide (BAM) in drinking water should be below 0.1 µg/L. This is currently achieved by activated carbon filtration. A more economical approach is bioaugmentation of sand filter units in drinking water production plants with *Aminobacter sp.* MSH1 that uses BAM as a sole source of C, N and energy.
Objectives: Successful bioaugmentation depends on both biotic and abiotic factors. Amongst those, interactions with and diversity of the indigenous microbial community are suggested to play a major role. To investigate the fundamental principles behind this hypothesis, MSH1 was combined with synthetic microbial communities and resulting functionality and survival of MSH1 were determined.
Materials & methods : Several bacterial strains were isolated from different sand filter samples. Combinations of these were co-inoculated with MSH1 in mineral medium with acetate as the sole carbon source in sterile sand. ^{14}C-labelled BAM was added and produced $^{14}CO_2$ was monitored. To quantify the success of bioaugmentation, survival of MSH1 was determined and kinetic parameters were derived from cumulative mineralization curves.
Results: First, a general diversity experiment was performed by increasing richness of sand filter isolates (SFI) at maximal evenness. Richness most distinctly affected mineralization rate, which was negatively correlated with richness and positively correlated with survival of MSH1.
Second, identity effects were traced by applying all possible richness 1 and 2 combinations using a total of 13 SFI. Certain SFI had an either positive or negative effect on invasion of MSH1 in all combinations, showing important interspecies interactions. At the time of presentation, more information will be available on how these species interact and a modelling framework will be set up to predict effects at richness 2 based on richness 1.
Conclusion: The use of synthetic microbial ecosystems has shown promising results in unravelling diversity effects and interspecies interactions, influencing the success of bioaugmentation and is of interest for its further optimization.

P CMC 5

A new molecular tool designed to evaluate the potential-emissivity of nitrous oxide by microbial ecosystems

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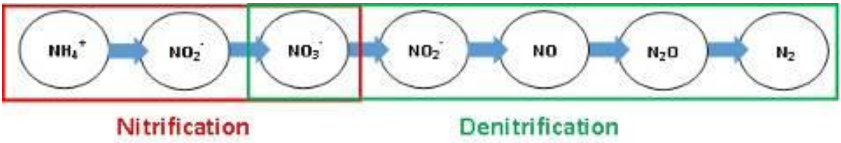
Climate change caused by greenhouse gas (GHG) emissions is one of the major challenges facing mankind today. Nitrous oxide (N_2O), which has a mean residence time of 120 years in the troposphere combined with a considerable ozone-destroying capacity in the stratosphere, has a global warming potential approximately 265-fold stronger than carbon dioxide, on a 100-year horizon (Ravishankara *et al.* 2009;IPCC 2013).
N_2O is a metabolite produced in both bacterial nitrification and denitrification pathways and can be naturally emitted by sols and oceans.A unbalance between N2O production and consumption rate leads to its release in the environment.
Fig: Nitrification and denitrification pathways
A genetic potential quantification for N_2O consumption has been described. This tool is based on the quantification by q-PCR of genes nir, encoding for nitrite reductase (NO_2), and noz, coding for nitrous oxide reductase (N_2O) (Smith and Osborn, 2009).The ratio of these two abundances translates a potential (presence or not of these genes in the ecosystem)and not an activity (transcription of this genes).
The aim of this work is to develop a reliable quantification tool of ecosystem metabolic activities driving to the accumulation of N_2O during heterotrophic denitrification. The measure an activity is based on the quantification by RT qPCR of the expression levels of genes nir and nos. This tool allows to assess the N_2O production levels of natural or anthropogenic ecosystems such as intensive and extensive wastewater treatment plants and soil .
Parameters of RNA extraction were performed on complex samples from different biological wastewater treatment processes. Primers sets targeting 3 genes implicated in the reduce chain of nitrates in nitrogen gas were targeted (nirK, nirS, nosZ), as wel las a reference gene(gyrA) to realise an absolute quantification of mRNA produced in these ecosystems.

Primers sets were tested on 6 denitrifying bacterial collection strains. All parameters of this 4 RTqPCR allowed to quantify the genetic activity of an ecosystem producing of N_2O.This tool was then tested on different denitrifying ecosystems.
These molecular tools appear to be relevant and very informative to describe the ability of various ecosystems to produce N_2O.

Figure 1



P CMC 6

Construction of synthetic microbial consortia for the bio-conversion of pig slaughterhouse keratin wastes into high quality feed

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Keratin wastes from pig slaughterhouses (e.g. bristles and hooves) constitute a potential source of protein presently poorly explored. The problem with keratin is its inherent resistance to biological degradation. Today keratin wastes are treated by costly thermochemical methods, generating low quality nutrients used in animal feeding, if not incinerated or dumped. The goal here is to convert keratin wastes into valuable products such as high quality feed enriched with short chain peptides and essential amino-acids using synthetic microbial consortia. Full biological degradation of keratin is possible, but the process is slow and single species cultures are known to lack robustness in case of phage infections or contaminations. We hypothesized that efficient degradation requires the concerted action of a stable microbial consortium working synergistically. Identifying such microbial consortia will provide efficient, cheap and sustainable ways to convert keratin wastes whilst increasing nutritional value. Several environments suspected to host keratin degraders were enriched in microcosm for selecting fitted and stable consortia displaying high keratin turn-over. The consortia were characterized using 16S rRNA amplicon sequencing coupled to meta-omic approaches. We also developed a reliable colorimetric assay for assessing and screening the keratinase activity of enriched consortia based on raw keratin waste labelling. In addition, a collection of 500 isolates was established and screened in order to provide good candidate members for potential construction of synthetic microbial consortia. Results are proving the approach to be successful toward selecting a wide panel of very different keratinolytic consortia harboring reduced diversity, with discovery of relevant genes encoding enzymes involved in keratin degradation.

P CMC 7

Construction of synthetic actinobacterial communities for oil-contaminated soil and water bioremediation

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Oil-contaminated soil and wastewater present one of the hardest environmental problems caused by oil industry. Oily wastes are extremely complex mixtures of hydrocarbons and their derivates with various bioavailabilities and biodegradabilities. Hydrocarbons-oxidizing actinobacteria of the genus *Rhodococcus* are biotechnologically promising microorganisms suitable for biodegradation of recalcitrant oil constituents, e.g. PAH and heterocyclic compounds. Upon revealing new catabolic abilities of *Rhodococcus* species, these bacteria have been increasingly explored for bioremediation of contaminated soils, waters and air [1]. Many papers showed that microbial consortia consisting of two or more strains exhibit better biodegradation performance than single strains [2]. Various co-cultures of *Rhodococcus* species alone or in combinations with other bacterial/fungi strains are used for bioremediation of contaminated environments [1]. However, sometimes mixed bacterial cultures show less efficient biodegradation compared to single strains presumably due to strain competition or antagonistic effects. We constructed actinobacterial consortia from *Rhodococcus* (*R. erythropolis, R. opacus* and *R. ruber*), *Dietzia maris* and *Gordonia rubripertincta* strains differing in substrate spectra and functional (biodegradation, adhesion, biosurfactant-production etc.) genes. Metabolic interactions within the communities were identified regarding the target function - model oil biodegradation, using a function-interaction modeling [3]. Selected efficient consortia were immobilized into poly(vinyl alcohol) cryogels [4] and tested in two industrially important processes: (i) bioreactor treatment of oil drilling wastewater and (ii) bioremediation of crude oil-contaminated soil. We assessed a survival and biodegradation activities of introduced consortia, as well as their interactions with intrinsic microbial communities.

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P CMC 8

Innovative washing solution based on *Lactococcus lactis*, nisin producing strain, and thyme essential oil at industrial level to improve safety and quality of minimally processed lamb's lettuce

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Disinfection processes incorporating chlorine are often applied to fresh vegetables in order to enhance safety and shelf-life profiles. However, the use of chlorine based processes are unable to guarantee the safety of minimally processed vegetables due to its low efficacy against pathogenic and spoilage microorganisms. Moreover, chlorine usage implies the possible formation of carcinogenic chlorinated compounds and vapors having adverse health effects as well as the increase of microbial chlorine resistance. For these reasons the use of chlorine is prohibited or restricted in some European countries for the disinfection of the raw materials used for the production of minimally processed vegetables. Plant essential oils, their components and biocontrol cultures were proved as alternative tools to chlorine to control foodborne pathogens without detrimental effects on shelf life and sensorial properties of minimally processed lamb's lettuce at lab scale level. The best performances were demonstrated by a selected culture of *Lactococcus lactis*, nisin producer, and thyme essential oil added alone or in combination, respectively at 6 log CFU/ml and 250 ppm, in the washing solution of lamb's lettuce. The innovative solutions were scaled up at industrial level in comparison with chlorine disinfection processes (120 ppm). The products were handled and packed following the standard company procedures and stored at 6°C. During the storage the quality parameters and the shelf life were determined in relation to the washing solution applied. In addition the effects of the biocontrol culture and/or thyme essential oil addition on lamb's lettuce microbiota were studied by culture dependent and independent methods (pyrosequencing). The results obtained showed no significant differences of the cell loads of mesophilic aerobic bacteria, yeasts and total and fecal coliforms in relation to the washing solution adopted. No significant

differences were also recorded for the color and texture parameters considered. By contrast the use of biocontrol agent and/or the thyme essential oil determined significant spoilage population shifts and significantly affected the volatile molecule profiles and the sensory features of the products.

P CMC 9

Towards an understanding of the sensitivity of pesticide degradation to losses of soil microbial diversity

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Soil microbial communities are highly diverse and implicated in delivering many ecosystem services including the *degradation* of *pesticides*. However, evidence is increasing that field soils, once sampled, processed and incubated according to OECD guidelines are less microbially diverse than field soils *in situ*. In addition, field soils undergo regular perturbation and recovery, which may impact microbial diversity and pesticide biodegradation. For many pesticides significant lab-to-field and soil-to-soil variability of degradation rates (DT_{50}) has been reported, but the relationship between microbial diversity and pesticide biodegradation kinetics has not yet been examined.
This study explores the sensitivity of pesticide biodegradation to microbial diversity erosion and aims to better understand the causes of DT_{50} variability.
Soil mesocosm series encompassing a microbial diversity gradient were created using a dilution to extinction approach. Mesocosms of fresh and sterile soil were included as controls. Following a five month equilibration, measurements of microbial biomass (SIR) showed that the mesocosms had reached similar levels of bioactivity. OECD307-compliant aerobic soil degradation studies of four model pesticides (2,4-D, Terbuthylazine, Azoxystrobin, Bicyclopyrone) were conducted using these equilibrated soils.
Analysis of compound degradation rates revealed pesticide-specific sensitivity to microbial erosion as well as clear differences in the patterns of response. The microbial 2,4-D degradation function was robust up to 10^{-6} dilution, but was disrupted in highly impoverished systems. Conversely, Azoxystrobin half-life was much prolonged (>417 days) in all diluted mesocosms compared to the 'fresh control' DT_{50} of 77 days, indicating a high dependency on microbial diversity. Degradation of Terbuthylazine did not follow the trend observed for 2,4-D and Azoxystrobin.
Data so far indicates that this test system is suitable for studying the impact of microbial diversity erosion on pesticide biodegradation. Our findings suggest that structurally different chemicals respond differently to microbial diversity erosion. Differences in microbial diversity may explain soil-to-soil variation in the field and lab-to-field variation in pesticides kinetics frequently observed.

P CMC 10

From Mouth to Model: improving methods for oral biofilm analysis

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Experimental studies of biofilm forming bacterial communities require in vitro simulations of native conditions. Yet, most of these approaches rely on biofilms composed of one, or only few, bacterial species, which does not properly reflect the complexity of natural systems. Oral biofilms comprise hundreds of species at different abundance. Ideally, an undisturbed transfer of the native bacterial community from the mouth to the lab is required. Our aim was to develop a workflow to grow native oral biofilm *in vivo*, transfer it to the laboratory and incubate it in biofilm reactors. Survival of native oral biofilm under laboratory conditions and changes of bacterial composition over time were observed. Standardized human enamel-dentin disks embedded in oral splints were used as biofilm carriers. Six disks were fixed on the inside of the splint facing the teeth buccally. Appliances were fitted individually to the lower jaw of 20 male non-smoking volunteers. Splints were worn for 48h continuously. Enamel-dentine disks were taken out and placed into biofilm reactors. Fed with BHI medium biofilm was incubated for another 48h *in vitro*. Live-dead staining was performed and evaluated with confocal laser scanning microscopy to monitor survival rates of the biofilm directly at three time points. Changes of the bacterial diversity over time were analyzed by 454 pyrosequencing. Survival curves started with an increase of bacterial numbers 1h after incubation and then decreased to and reach the initial level after 48 hours. Compositional shifts during *in vitro* growth were revealed with 454 pyrosequencing comparing t0 and t3. Major phyla found were *Firmicutes* (86.58%), *Bacteroidetes* (5.34%), *Proteobacteria* (3.99%) and *Actinobacteria* (1.21%). *Fusobacteria* (0.64%), *Cyanobacteria* (0.58%) and *TM7* (0.1%) represented groups

around 0.1%. Bacterial diversity decreased over 48h but was not lost completely. *Streptococci* dominated the biofilm with 60% at the start going up to over 90% in some samples at t3. *Clostridia* also multiplied strongly. Environmental variables such as temperature shifts, changes in nurture, interaction with the host and aggressions from external bacteria are likely to have further impact on bacterial survival and oral biofilm diversity.

P CMC 11
ASSESSING THE SOIL MICROBIAL INTERACTOME
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Microbial ecosystem engineering approaches often rely on the introduction of one or more selected species into an existing microbial community. The success of introduced species may to a large extent depend on the types of interactions that the microorganism is developing with other existing microbes, such as neutralism, commensalism, syntrophism or competition. Deciphering the rules governing such organised establishments is a strenuous task.

Our project is inclined towards underlining the principles of success of establishing pure cultures in complex microbial ecosystems such as contained within soil. Here we focus on the development of a high-throughput co-cultivation approach that might enable us to study the species "interactome", the identification of species combinations that decide favourable/non-favourable community fitness. The on-going study involves the use of agarose micro-beads as growth chambers. The co-cultivation technique immobilises cells allowing species-species interactions to be studied by microscopy and flow cytometry. Possible negative or positive species interactions can then be studied in more detail by sorting or separating beads of interest. The resulting knowledge not only provides ample data in designing functional synthetic communities but also construct new avenues for “synthetic ecology”.

P CMC 12
Studying the Effect of Increasing Micro-Predator and Prey Diversity on Wastewater Pathogen Removal in a Miniature Membrane Bioreactor System
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Question: The permanent availability of clean water is taken for granted in most Western countries. However, water shortage is a growing concern worldwide and a main issue in Israel and Palestine. An approach to overcome this problem might be the exploitation of predator-prey interactions to reduce pathogens in waste water more efficiently.
Methods: In this trilateral study we investigated the impact of the diversity of micro-predators on pathogen removal in highly controlled small scale systems. For this purpose, we assembled different predator-prey combinations in miniature membrane bioreactors (mMBR) functioning as laboratory-size wastewater treatment plants (WWTP) over a four day period. Predators included protists (generalist predators), bacteriophages (specialist “predators”) and specific predatory bacteria (*Bdellovibrio*-and-like organisms, BALOs) that can only prey upon gram negative bacteria. *Klebsiella sp*., *Staphylococcus sp*., and *Pseudomonas putida* were included as typical wastewater pathogens.
Results: Only the specialized predators were able to drive their preferred pathogen to extinction. In contrast, the generalist predator lead to a steady reduction of all pathogens. All predator and prey species were able to coexist together in a reactor. We also found that predator growth was higher when incubated with multiple prey species, independent of their ability to utilize all those species. At the same time, the reduction of the preferred prey organism was higher. Additionally, changes in the generation times of the pathogens were observed. Interestingly, the addition of the specialized predators led to a significant decrease in the pathogens generation time. In contrast, protist predation led to an increase in the pathogen generation time, although not significantly.
Conclusion: We believe that studying the effect of microbial diversity across trophic levels in highly controlled and reproducible laboratory systems will answer central questions in microbial ecology and predator-prey-theory, and might lead to an improvement of the current technology. In the future, this might result in a better management of microbial resources and in a more efficient reduction of pathogenic bacteria during the wastewater treatment process.

P CMC 15

Ecology and evolution of kin recognition in *Bacillus subtilis*

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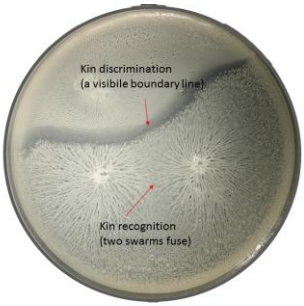
Background: Microbial social interactions have either positive (cooperative), negative (antagonistic) or neutral consequences. How interacting microorganisms decide between these opposing behaviours? Hamilton theory states that cooperative acts are preferentially directed towards relatives. Therefore, bacterial populations must have evolved an ability to recognize their relatives (kin).
Objectives: We aimed to determine the distribution and mechanisms of kin recognition in natural *Bacillus subtilis* populations isolated from soil microscale by using the collection of 39 *Bacillus subtilis* isolates that have a history of coexistence in soil.
Methods: We determined relatedness of strains by sequencing housekeeping genes and then analysed more than 700 strain combinations for kin recognition (fusion of swarms) or discrimination (formation a visible boundary line between swarms) phenotypes. We also addressed kin recognition mechanisms by screening *Tn* mutants and the parental strain combinations.
Results: We found that swarms of the same strain recognize each other as kin but below 99.8% identity kin recognition is lost ultimately giving 13 kin recognition groups among 39 *B. subtilis* isolates. This striking phenomena was observed on nutrient agar and on plant roots where kin strains coexisted while non kin competed each other. Several genetic loci encoding socially important traits were identified as candidates for kin recognition/discrimination mechanisms.
Conclusions: We show for the first time the existence of kin recognition in natural population of *B. subtilis* isolates. This ability is lost early during evolutionary divergence of clonal groups and involves several genetic loci suggesting multiple mechanisms directing kin recognition. The ecological role of kin recognition may be to 1) stabilize intra-clonal cooperation and 2) minimize the invasion by non-kin neighbours, constraining their antagonistic acts to the boundary line.

Figure 1



P CMC 16

Temporal-niche partitioning of key species and their functions in a 14-year anaerobic benzene-degrading bioreactor

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Development and testing of novel microbial ecology theories has been hampered by the almost immeasurable diversity found in most natural environments. Due to the complexity of interactions found in such systems, we are currently unable to determine and monitor all functions and key players. Constructed microbial communities, such as enrichment cultures, conserve complex functions and exhibit relatively simpler species diversity. We used a stable benzene-degrading anaerobic consortium to determine, monitor and model microbial functions in a complex system. Benzene, a monoaromatic hydrocarbon, is a model molecule to study conversion of aromatic compounds into non-hazardous substrates. Syntrophic interactions between microorganisms in mixed microbial consortia appear to be critical in the anaerobic degradation of benzene. Our benzene-degrading consortium has been kept for more than 14 years under continuous culture conditions. In this study, we performed a succession experiment in batch culture to resolve the occurrence of and interactions between species over time. The experiment was carried out for 34 days, during which we sampled at 8 different time points, obtaining a total of 120 samples. Community analysis, based on high-throughput sequencing of 16S rRNA genes, revealed 197 Open Taxonomic Units (OTUs) at 97% similarity ('species' level). The most dominant phyla were Proteobacteria (average, 70%) and Firmicutes (average, 24%). We integrated quantitative community composition data into co-occurrence network analyses. Results indicate temporal-niche partitioning. Species belonging to the family *Peptococcaceae* became dominant (45%) once benzene degradation started. Previous studies have indicated that this species ferments benzene. Currently, we are sequencing 12 metagenomes of different temporal niches to reconstruct genomes of the dominant species. This will allow us to supplement the network analysis with genome information, thus to link phylogeny to potential functions and metabolic interactions in the consortium. An ecological understanding of interactions between *Peptococcaceae* and other members of this community will help to develop strategies to control functions in our consortium, and to extent this knowledge to remediate benzene pollution *in situ*.

P CMC 17

A novel high-throughput drip-flow system to grow autotrophic biofilms of contrasting diversities

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The impact of community diversity on the functioning and assembly of microbial systems remains a central questions in microbial ecology. This question is often addressed by either combining a few cultures without necessarily a history of coexistence, or by using environmental communities, which are often ill controlled and thus likely to be poorly reproducible.
The purpose of this work is to develop a high-throughput continuous-flow system for growing replicate microbial biofilms of varying, but controlled, average thickness and associated community diversity. With these replicate biofilms, the effect of community composition and diversity on various ecological processes can then be rigorously examined. We hypothesize that the increased loading, resulting in thicker biofilms, will decrease the drift in the community and impose limited environmental filtering by providing more diverse niches. Thus, thicker biofilms are likely to host greater diversity.
A system with 40 replicates has been constructed using flow-through polypropylene columns housing a defined number of single-sized glass beads supported by a stainless steel mesh. Biofilms consisting primarily of ammonia oxidizing and nitrite oxidizing bacteria are cultivated on the beads using a drip-flow assembly by feeding a mineral medium containing ammonium-N as sole energy source. Biofilm thickness is controlled by setting the surficial loading rate to 0.168 g NH_4-N/m^2/day or 1.678 g NH_4-N /m^2/day, which should theoretically result in biofilms with average thickness of 100 or 1000 µm.
We will present the differences observed in community composition between systems run at high and low loading rates for 60 days. We will also evaluate community activity by measuring nitrification efficiency and correlate that to microbial diversity.
In conclusion, we hope to demonstrate a high-replicate biofilm cultivation systems that allow us, by altering the loading rate, to engineer biofilms towards prescribed differences in composition, opening new opportunities to explore community assembly processes and their link to ecosystem function.

P CMC 18

Microbial consortia bred from soil on different lignocellulosic substrates reveal distinct players acting in lignocellulose degradation

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Plant biomass constitutes a valuable source of energy, however its industrial degradation is not yet fully established. Here, we bred microbial consortia from forest soil using wheat straw (pH 7.2 and 9.0), switchgrass (pH 7.2) and corn stover (pH 7.2) as carbon sources to investigate how different lignocellulosic substrates and pH conditions structure the microbial communities (bacterial and fungal) along aerobic sequential-batch enrichments. Nine sequentially transferred cultures were evaluated by quantitative PCR and PCR followed by denaturing gradient gel electrophoresis (DGGE) to measure the abundance, stability and structure of microbial communities. Along all cultures, bacterial growth was achieved, from initially ~10^5 to finally ~10^8 cells per ml. PCR-DGGE profiles showed that the sequential-batch transfers reached stabilization at transfers 4 to 6. The microbial communities were consistent between replicates per substrate, yet differed up to 40% (Bacteria) and 60% (Fungi) among substrates. This indicated a microbial core shared across substrates in addition to a variable (substrate-specific) microbiome. Thus, microbial communities bred from a unique inoculum source on different pH and lignocellulose sources, consistently yield different community structures excepted that pH did not strongly affect the fungal communities. To understand the substrate degradation at the organism level, 36 bacterial and 13 fungal strains were isolated during the experiment. Strains affiliated to *Sphingobacterium, Raoultella/Klebsiella, Pseudomonas, Stenotrophomonas,* (Bacteria) *Coniochaeta,* and *Acremonium* (Fungi) were recovered in at least three treatments, suggesting that can be part of the microbial core able to deconstruct the plant biomass, as was indicated by CMC-ase and xylanase activities. Interestingly, *Delftia, Paenibacillus, Sanguibacter* and *Comamonas* strains were recovered only in SG or CS, suggesting that these are specialist microorganism according to the substrate used. This study highlighted the importance of an efficient substrate usage, as well as pH condition, in microbial consortia enrichment to develop lignocellulose degradation application.

P CMC 19

Bacterial community structure changes during colonization and fruiting body production of oyster mushroom using a composted natural substrate

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Oyster mushroom (*Pleurotus ostreatus*) is a saprotrophic, edible, white rot fungi. In Europe it is grown on composted and pasteurized wheat straw (substrate) with Actinobacteria, Firmicutes and *Thermus* spp. as the dominant bacteria identified recently using Sequence-aided T-RFLP (Terminal Restriction Fragment Length Polymorphism). Bacteria in the mature substrate hinder the colonization of competing and pathogenic microbes of *Pleurotus* sp. However their interactions with oyster mushroom during its substrate colonization are not yet clarified. As a first step, in this study we wanted to monitor the changes of bacterial community as an impact of the oyster mushroom substrate colonization and fruiting body (FB) production.

The 10-week-long oyster mushroom cultivation, carried out at a mushroom farm consisted of two cycles of substrate colonization and FB production. Bacterial community changes were monitored weekly in five substrate blocks with 16S rRNA gene based T-RFLP and selected samples were subjected to NGS (Next Generation Sequencing) pyrosequencing analysis. The activity of *Pleurotus* sp. was characterized by its lignocellulose degrading enzymatic activities.

Bacterial community showed a clear parallel succession in the five substrate blocks forming 4 groups according to the four consecutive phases of mushroom production. Oyster mushroom had high laccase activity during initial colonization, having presumably impact on bacterial community, while level of manganese-peroxidase and cellulases increased during FB induction and FB formation. Bacterial community was predominated from the beginning by the Firmicutes phylum (*Bacillus* spp. and related species) with continuous increase. Also the ratio of the halotolerant *Halomonas* spp. increased. Parallel members of Actinobacteria and *Thermus* spp. disappeared from the community.

In the near future the absolute amount and activity of Firmicutes bacteria should be analysed to reveal whether they just survive or they have an active role in *Pleurotus* production.

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P CMC 20

Measuring Patterns by Geographical Locations in Marine Metagenome Data using Newly Adopted Genotyping by Sequencing

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Introduction: As handling of the huge quantity of metagenomic data is difficult and time-consuming, we proposed more effective metagenomic tool to analyze particular sequences that related restriction enzyme sites for targeting reduced numbers of genes than to estimate whole genome sequences. This is adapted from a novel approach, Genotyping-by-sequencing (GBS) procedure in plants for analyzing marine metagenome.
Objectives: In this study, single nucleotide polymorphisms (SNPs) in restriction enzyme associated sequences by environmental changes were compared by gene's functional categories.
Materials & methods: GBS-metagenome procedure was demonstrated with marine epipelagic samples collected on 10 locations across from East Sea to Bering approximately 8,000 km apart on July, 2013. These samples were processed by a restriction enzyme *ApeKI* and then sequenced by Illumina HiSeq, yielding 74.4 million reads. These restriction enzyme associated sequences were grouped (clustered), aligned, and annotated using UCLUST, MUSCLE, and tBLASTP respectively. Entropy in sequence variation among sorted clusters by functional category was measured to evaluate their rate of SNPs variations.
Results: Among the 10 sites, there were the difference of entropy score in clusters assigned to rhodopsin, ABC transporter, and metabolic genes between East Sea (west side of Japan) and Western Pacific Ocean (East side of Japan). Higher entropy values in Western Pacific Ocean than those in East Sea may represent the genetic difference by environmental alteration, geographical location and the influences of Cs-137 radioactivity after Fukushima Daiichi nuclear disaster.
Conclusion: GBS-metagenome could identify nucleotide variations for describing significant differences between open and closed ocean in each functional groups through comparing each site and more. Impacts of nucleotide variants by ^{137}Cs radiation should be verified through further studies with comparing Shannon entropy score in detail.

P CMC 21

Dividing metabolic labor among microbial cells accelerates the consumption of substrates that produce growth-inhibiting intermediates

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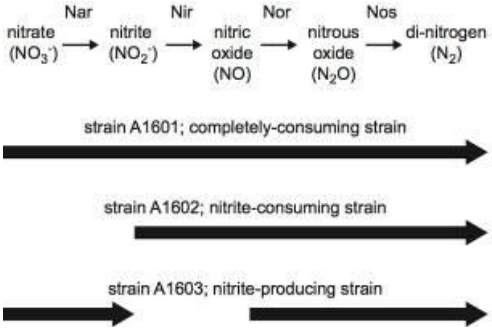
Question: The division of metabolic labor is a general principle that shapes the structure and functioning of nearly every microbial community. A canonical example is substrate cross-feeding, where one cell partially consumes a primary substrate into metabolic intermediates and other cells then consume the intermediates. While substrate cross-feeding often occurs, the consequences on substrate consumption are unclear. We hypothesized that the production of growth-inhibiting intermediates is one factor that determines whether substrate cross-feeding accelerates substrate consumption relative to complete consumption.
Methods: To test our hypothesis, we engineered synthetic communities from isogenic mutant strains of the denitrifying bacterium *Pseudomonas stutzeri* A1501. One strain completely consumes nitrate to dinitrogen gas, the second strain consumes nitrate to nitrite, and the third strain consumes nitrite to dinitrogen gas. We grew the first strain alone or the latter two strains together in nitrite cross-feeding consortia and measured the speed at which nitrogen oxides are consumed. We manipulated the inhibitory effects of nitrite by adjusting the pH of the culture medium, where the inhibitory effects increase as the pH decreases.

Results: We demonstrate that the inhibitory effects of nitrite determine whether nitrite cross-feeding accelerates substrate consumption relative to complete consumption. We further demonstrate that this result emerges because nitrite cross-feeding reduces the accumulation of nitrite.
Conclusions: Our results provide experimental evidence that substrate cross-feeding does indeed accelerate substrate consumption when substrates produce growth-inhibiting intermediates. Knowledge about the inhibitory effects of metabolic intermediates might therefore be useful for deciding how best to distribute different metabolic processes across different cells to optimize a particular metabolic process.

Figure 1



P CMC 22
Combining microbial microcosms and mathematical models to enhance our quantitative understanding of diversity-disturbance relationships in ecology.
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Understanding the relationship between biological diversity and environmental heterogeneity is crucial for predicting ecosystem responses to natural and anthropogenic disturbances. The Intermediate Disturbance Hypothesis (IDH) posits that diversity peaks at intermediate disturbance and has guided diversity-disturbance work for decades. However, empirical support for the IDH is mixed, in part due to the difficulties of experimentally manipulating macroecological systems. To overcome these challenges, we develop microbial microcosm experiments to test for the IDH over a range of disturbance dimensions. We find strong support for the IDH when disturbance is sufficiently coupled to environmental parameters. Based on our results, we formulate a Lotka-Volterra model to explore the underlying dynamics. Finally, we propose a non-equilibrium state-mixing model for the IDH, which offers insights into the timescales of diversity-disturbance relationships and the interaction between disturbance intensity and frequency. Together, we provide conditions that enable the IDH to serve as a useful, predictive ecological theory.

P CMC 24

Taxonomic affiliation of esterase and lipase sequences of a metagenomic library constructed from oil-impacted mangrove sediment

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Mangroves are rich ecosystems and constitute a habitat for organisms that are able to tolerate severe conditions. This environment has an intense biological activity and microbial communities play essential roles in its functioning and maintenance. In 1983, the mangrove of Bertioga city (Brazil) was affected by an oil spill, when 35 million liters of oil were released into the area. Bertioga microbial communities adapted to such harsh conditions over the years, thus providing an interesting source of potential active molecules. The aim of this work was to perform a taxonomic affiliation of microbial sequences related to esterases and lipases recovered in a mangrove sediment metagenomic library. A metagenomic library was constructed using the "Cloning-Ready Copy Control pCC2FOS" kit (Epicentre®) yielding 12,960 clones which had their fosmid DNA extracted (QIAGEN Large-Construct kit) and subsequently sequenced using Pyrosequencing (454 GS FLX Titanium technology), resulting in a total of 1,380,509 reads. Reads were trimmed and data were annotated using the MG-RAST pipeline V.3.3.8 (cut-off e-value of $1e^{-5}$, ID 4558576.3). Taxonomic affiliation of hydrolases sequences was first performed by searching for each enzyme using SEED and then using the results to run Best Hit Classification tool (M5NR). Taxonomic affiliation of esterase and lipase sequences showed that the majority of them belonged to Proteobacteria and Actinobacteria. For esterases and lipases, respectively, 5.48% and 5.74% of sequences originated from representatives of the Planctomycetes phylum. In addition, 4.46% of lipase sequences were affiliated to Acidobacteria phylum. Members of these two phyla are known to be difficult to isolate and little information is available about these groups. There is no metagenomic data available for comparison and as the esterases and lipases described so far belong to different phyla, the results obtained are innovative. Results may allow access to sequences and exploitation of retrieved data, enabling primer design for amplification of these enzymes and subsequent expression assays to verify their activities and sequence novelties.

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P CMC 25

***Arundo donax* hydrolysates shape hydrogen-producing microbial community in dark fermentation processs**

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Dark fermentation (DF) has great potential for development as a sustainable biohydrogen production system. Mixed anaerobic microbial consortia from sewage or wastewater sludge digester can be used as seeding for fermentative hydrogen (H_2) production. Biomass such as energy crops are good substrates for H_2 fermentation.
Arundo donax (L.), a perennial non-food crop with high biomass, was chosen as a source of lignocellulosic biomass in a pilot-scale DF process. A mixed microbial consortium from a primary sludge digester, adapted to a synthetic medium amended with glucose, was used as inoculum. Before starting the *A. donax* hydrolysates fermentation, the inoculum was acclimated either on glucose or on *A. donax* hydrolysates. A glucose fermentation with glucose-acclimated inoculum was used as control.
The microbial communities were characterized by Pyrotag sequencing of 16S rRNA genes. Hydrogen producing populations were quantified by Real-Time PCR of specific 16S rRNA and hydrogenase genes. Other coexisting microorganisms were also quantified. Performances of *A. donax* fermentation in presence of differently acclimated consortia were compared by analyzing molecular data in conjunction to H_2 production.
In glucose fermentation, where H_2 production was fast, the bacterial consortium was characterized by the co-dominance of Enterobacteriaceae and Lactobacillaceae being 80% and 15% of the total community respectively.
Microbial communities of both *A. donax* fermentations strongly differed from those of glucose-fermentation, being characterized by the dominance of Lactobacillaceae (40% of the total) and low percentages of H_2 producing populations (<0.5% of the total). Quantification of hydrogenase genes of *Clostridium* spp. confirmed that these populations were present in low amount (10^4 gene copies). Nevertheless, remarkable H_2 yields were recorded.

The study evidence that the inhibitory compounds derived from the hydrolysis of *A. donax* had a prompt effect on the microbial community of the inoculum. However, this noticeable shift in the microbial communities did not affect H_2 production. Finally, adaptation on different carbon substrates exerted a comparable selective pressure, leading to similar H_2 yields.

P CMC 26

Enrichment and development of a microbial community capable of Organic Municipal Solid Waste degradation and Ethanol production

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Lignocellulose derived ethanol (EtOH) is a promising sustainable alternative to fossil transport fuels. Currently, research has been focused on engineering "industrially friendly" microorganisms to hydrolyse cellulose and hemicellulose while fermenting the hydrolysis products to EtOH. This approach still faces instability, narrow operational conditions and is susceptible to contamination. A suitable alternative could be the use of a microbial community, where different organisms perform the required biochemical processes and the relationships between its members would provide resilience to the system (Zuroff and Curtis, 2012). The objective of this work is to develop a microbial consortium for the bioconversion of Organic Municipal Solid Waste (OMSW) to EtOH, from different environments where lignocellulose degradation is likely to occur naturally. This ongoing research involves the sampling of composting piles, where OMSW is already being aerobically degraded; forest soil, where the leaves-mat is degraded by soil microorganisms; rumen and cattle dung, where highly specialized cellulose degraders and anaerobic digestate where fermenters thrive. Inocula are cultured under anaerobic and aerotolerant conditions, with pre-treated (autoclaved 121°C, 15 min. with H_2SO_4 1% v/v) and raw OMSW analogue. Mixtures of the samples, one of them integrated with all the environmental sources, are also tested. The analysis of the consortia through molecular biology techniques will allow the comparison of their diversity, function and structure, to understand their differences and pinpoint the characteristics that make the most successful consortium (highest EtOH production) to thrive, to determine if there is a stable higher species diversity when sources are combined, as well as if functional redundancy is achieved. Ethanol is expected to be present at as a low-concentration in mixed culture fermentations, although efficient energy capture within the communities can lead to its oxidation. The results from these experiments along with those from chemical analysis will be the basis for the construction of a model of the system, aimed to direct and optimize the selected consortium activity towards EtOH production by manipulating environmental conditions (temperature, pH, oxygen concentration and hydrogen partial pressure).

Zuroff, T. and Curtis, W. (2012) 'Developing symbiotic consortia for lignocellulosic biofuel production', *Appl Microbiol Biotechnol*, 93(4), pp. 1423-1435.

P CMC 27

Screening of antibiotic producing actinomycetes from the sediments of undisturbed forest areas of Asella, Ethiopia and its hyper activity after mutation

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Wide and uncontrolled usage of antibiotics has made the pathogens to become resistant to currently used antibiotics. There is an urgent need for development of a new drug or a highly active molecule for controlling antibiotic resistant strains. In this study 32 strains of *Actinomycetes* were isolated and subjected to primary screening by giant colony method against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Aeromonas hydrophilia*. The secondary screening was carried out by fermentation process. Antibacterial activity was evaluated by well plate method. The extract of isolates was subjected to well plate method against pathogenic bacteria. *B. subtilis* and *E. coli* was highly inhibited by R1 isolate. Other isolates showed limited inhibition of bacteria. The R1 isolate was mutated by UV irradiation. The mutants differed from the wild parent in reduced growth rates, changes in the shape and size of the colony, sporulation level, antibiotic activity and variation in the color of the mycelium. *Bacillus subtilis* and *E. coli* was highly inhibited by AK1 isolate. Comparatively the zone of inhibition was higher with *Actinomycetes* AK1 inoculated plates. The secondary metabolite production was enhanced by UV mutagenesis when compared to wild type. Actinomycetes may produce different molecule that can inhibit different types of pathogens, however efforts like strain development can be done to produce new bioactive components against multidrug resistant bacteria.

P CMC 28

Responses of synthetic microbial communities perturbed in multiplexed continuous bioreactors as an ecologically-relevant proxy for microbial robustness prediction in natural and engineered ecosystems

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Biological robustness is a key attribute of biosystems that guarantees the maintenance of structural organization and/or function performance in the face of disturbances and uncertainty. Particularly, microbial ecosystems exhibit specific robustness attributes arising from the assembly and interaction networks of diverse, heterogeneous communities challenged by fluctuating environmental conditions. Stability, resistance and ecological resilience of natural, host-associated and engineered microbial communities rely on specific trade-offs between modularity, taxonomic and functional diversity and redundancy. Because of the complexity of naturally-occurring microbial systems, synthetic ecology, i.e., the rational design and theory-driven manipulation of artificial microbial ecosystems, is highly relevant to decipher key biodiversity-stability relationships thanks to the analysis of replicate systems of lower complexity and higher controllability and reproducibility in the presence of specific perturbations. Here, we present the design and construction of multiplexed continuous bioreactors used as a microbial cultivation and perturbation platform for the assessment and prediction of microbial community robustness. Continuous culture systems can provide several advantages over batch systems, such as (i) perturbation experiments using microbial communities maintained under controlled and environmentally-relevant conditions (e.g., oligotrophic environments) and (ii) relevance to meta-omics approaches that require data obtained under rigorously defined and regulated conditions. The multiplexing of chemostats enables the simultaneous realization of biological replicates with appropriate controls to compare disturbance responses of planktonic cultures using sustained selective pressure (press disturbance), pulse perturbation, or both.

Poster presentations

P FMC 1
Bacterial and fungal organisms associated with dining tables in South East Nigeria, West Africa

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Swab samples were collected from 10 restaurants in Okigwe and Abia state University Uturu, South-east Nigeria. The bioload as well as the different contaminating bacteria and fungi were studied to know the organisms that can be found on the surfaces of these dining tables. These organisms were identified by using standard microbiological techniques such as the API CH 20. The results obtained showed that Bacillus cereus, Salmonella typhi and S. paratyphi, Staphylococcus aureus, Streptococcus faecalis, Shigella flexneri, Escherichia coli, Mucor mucor, Penicillum sp. , Aspergillus niger and Alternaria sp. were the major bacteria and fungi found. Salmonella spp. and Staphylococcus aureus were the most prevalent bacteria. Among the fungal isolates, Aspergillus niger had the highest prevalence rate. These microrganisms isolated and studied are of serious public health importance and requires serious attention too. They also form an important micro biome in these extreme environments were they occupy some micro niches.

P FMC 2
Aqueous two phase extraction of *Jonesia denitrificans* xylanase 6 in PEG 1000/phosphate system.

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The impetus for research in the field of bioseparation has been sparked by the difficulty and complexity in the downstream processing of biological products. Indeed, 50% to 90% of the production cost for a typical biological products resides in the purification strategy. There is a need for efficient and economical large scale bioseparation techniques which will achieve high purity and high recovery, while maintaining the biological activity of the molecule. One such purification technique which meets these criteria involves the partitioning of biomolecules between two immiscible phases in an aqueous system (ATPS). The Production of xylanases is carried out in 500ml of a liquid medium based on birchwood xylan. In each ATPS, PEG 1000 is added to a mixture consisting of dipotassium phosphate, sodium chloride and the culture medium inoculated with the strain *Jonesia denitrificans*, the mixture was adjusted to different pH. The concentration of PEG 1000 was varied : 8 to 16 % and the NaCl percentages are also varied from 2 to 4% while maintaining the other parameters constant. The results showed that the best ATPS for purification of xylanases is composed of PEG 1000 at 8.33%, 13.14 % of K_2HPO_4 , 1.62% NaCl at pH 7. We obtained a yield of 96.62 %, a partition coefficient of 86.66 and a purification factor of 2.9. The zymogram showed that the activity is mainly detected in the top phase.

P FMC 3
An antifungal peptide from actinobacteria (*Streptomyces* sp. TKJ2) : Isolation and partial characterization

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Actinobacteria are of special biotechnological interest since they are known to produce chemically diverse compounds with a wide range of biological activity. This distinct clade of Gram-positve bacteria include some of the key antibiotic producers and are also sources of several bioactive compounds, established commercially A newly filamentous bacteria was recovered from Tikjda forest soil (Algeria) for its high antifungal activity against various pathogenic and phytopathogenic fungi. The nucleotide sequence of the 16S rRNA gene (1454 pb) of *Streptomyces* sp. TKJ2 exhibited close similarity (99 %) with other *Streptomyces*16S rRNA genes. Antifungal metabolite production of *Streptomyces* sp TKJ2 was evaluated using six different fermentation media. The extracellular products contained potent antifungal agents. Antifungal protein produced by *Streptomyces* sp. TKJ2 on PCA medium has been purified by ammonium sulfate precipitation, SPE column chromatography and high-performance liquid chromatography in a reverse-phase column. The UV chromatograms of the active fractions obtained at 214 nm by NanoLC-ESI-MS/MS have different molecular weights. The F20 Peptidic fraction obtained from culture filtrat of *Streptomyces* sp. TKJ2 precipitated at 30% of ammonium sulfate was selected for analysis by infusion ESI-MS which yielded a singly charged ion mass of 437.17 Da.

P FMC 4

Magnetic in situ hybridization (MISH) combined to specifically adapted microfluidics as a complementary approach to metagenomics

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Metagenomics based studies in complex environments such as soils are compromised by the enormous microbial diversity level. Even the higher throughput sequencing techniques applied to soil extracted DNA are unable to detect bacterial populations other than the most abundant ones missing the so-called rare biosphere. In addition, the high complexity of the metagenome prevents complete bacterial genomes to be efficiently reconstructed from soil sequences in contrast to less diverse ecosystems.
A complementary approach to metagenomics can be proposed based on the fractionation of the biodiversity for investigating bacterial populations separately and successively after their cells have been specifically extracted out of the environmental sample.
We developed an *in situ* hybridization based approach in order to separate specific bacterial populations out of a complex microbial community. The technique uses magnetically labelled polynucleotide probes targeting a hypervariable region of the 23s rRNA to hybridize the cell suspension recovered from soil by the nycodenz extraction protocol prior the labelled cells are trapped on a micro-magnet network inside a microfluidic device.
In our talk, we will show and discuss results obtained under controlled experimental conditions that demonstrate the potential and the limit of the approach and preliminary data of an application of the tool to trap indigenous soil bacterial populations.

P FMC 5

Exploring diverse environments using metagenomics for novel natural products

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There is an urgent need to discover and develop new compounds with novel antimicrobial activity due to the continued increase in resistance to multiple antibiotics seen within bacteria. In the environment the production of secondary metabolites is often correlated with polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) genes, which are present in a diverse range of bacteria constituting an interesting target for metagenomic studies. The aim of this work is to identify environments with potentially diverse secondary metabolites using next generation sequencing technologies and exploitation of the unculturable fraction of microbial communities creating metagenomic libraries. Different environments including Cuban, Algerian, Antarctic and European soils were studied, using Illumina MiSeq technologies, specifically looking for 16S rRNA and secondary metabolite (PKS and NRPS genes) diversity. Results showed a correlation between secondary metabolite diversity and different microbial communities present in the environment, with the most unique being in the Antarctic community. Two metagenomic libraries were created in fosmid vectors from Antarctic and Cuban soils and a PCR screening was performed with newly designed degenerate primers for PKS and NRPS genes identifying a total of 21 positive clones. Sequence analysis revealed that although clusters seem potentially novel, they are likely to be partial; therefore further libraries will be created in an engineered BAC vector to capture the whole gene cluster. The expression of some of these clones is currently performed in different heterologous hosts such as *Streptomyces coelicolor* M1152 and *Pseudomonas* in order to further characterise the compounds using high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS).
In conclusion, these preliminary studies on diverse environments showed the potential of metagenomics for novel natural product discovery.

P FMC 6

Long-Term Cultivation of Soil Microorganisms in Nanoliter-Droplet Arrays

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Soil communities are highly dynamic system, which are modulated in their composition and activity by physical and chemical factors [1]. New technologies are required for efficient screenings with respect to these large parameter spaces. One crucial problem is the separation of cultivation spaces. Beside micro segmented flow [2], droplet-based techniques are well suited for cultivation in parallel, but suffer from the droplet/droplet contact and the risk of cross-talk between the microfluidic compartments.
Here, a carrier for screenings in droplets with volumes in the lower nanoliter range was tested for the cultivation of soil bacteria. The carrier ensures the separate positioning and storage of droplets for longer incubation time without uncontrolled droplet motion and interaction, in contrast to emulsion techniques. The carrier in microscopy slide-format contains 2592 droplet trap positions, subdivided in 24 chambers. Its transparent material allows an easy microscopic monitoring. It is suited for investigation of single species as well as for the investigation of the growth behaviour of microorganism communities from soil samples. The droplets with a volume of about 4 nL are stable during cultivation times of up to 12 weeks, and therefore suited for stochastic confinement and cultivation of slowly growing soil bacteria, too.

Acknowledgement: For cooperation and stimulating discussions we thank K. Martin, M. Rothe and E. Kothe (Jena). The financial support of BMBF (PTJ, Bactocat, Kz. 031A161A) is gratefully acknowledged.

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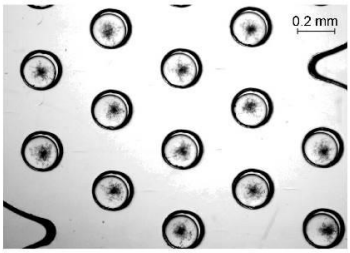
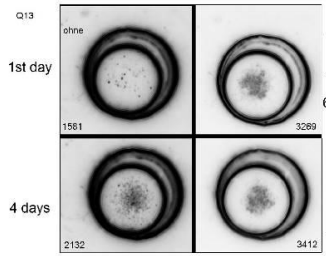
Figure 1



homogeneous growth
of soil bacteria
in nanodroplets
(streptomyces)



growth monitoring of
unknwon bacteria
from a soil sample
in nanodroplets

P FMC 7

Metagenomic analysis of the acid mine drainage from gold mine in Western Siberia revealed novel bacterial lineage

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Acid mine drainage (AMD) is an outflow of water from metal or coal mines. Such waters are typically highly acidic due to the metal sulfide oxidation and contain elevated concentrations of metals (iron, aluminum, zinc and others) and metalloids. Such environments are hostile to most life and usually harbor relatively simple microbial communities. We analysed the microbial community of acidic (pH 2.3, temperature +26°C) puddle at the tailings, containing solid wastes from Centralnaya gold mine, Kemerovo region, Russia. We used the pyrosequencing the total community DNA to analyse the community structures and to assembly draft genomes of the dominant species. Although AMD environments are relatively well studied, the most abundant group (relative abundance about 60%) was represented by a novel uncultured lineage only distantly related to delta-proteobacteria with 84% 16S rRNA sequence identity to the closest cultured bacterium, *Syntrophus aciditrophicus* SB. Most other bacteria represented known cultured groups previously found in AMD, - *Acidithiobacillus* (17%), *Ferrimicrobium* (5%), *Leptospirillum* (5%), *Acidisphaera* (4%), and *Acidibacter* (2%). Two groups of archaea, both representing euryarchaeota, were found, namely *Ferroplasma acidarmanus* (3%) and a novel lineage distantly related to *Thermoplasmatales* (92% 16S rRNA identity with *Thermoplasma volcanium*). The near-complete genome of the dominant novel uncultivated lineage was reconstructed. Genomic analyses of this organism reveals heterotrophic lifestyle. This work was supported by the Russian Science Foundation (project 14-14-01016).

P FMC 8

Ecological diversity of the genus *Acinetobacter* and its role in genetic diversification and emergence of pathogenicity

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Bacterial comparative genomics has increased our understanding of bacterial functional diversification within a species. To understand multiple independent emergence of pathogens in certain bacterial clades such analyses must be made at larger taxonomic levels. We have therefore sequenced all known and most proposed new species in a single genus - the *Acinetobacter*. In this genus, *A. baumannii* is a major nosocomial, but other species are emerging as opportunistic pathogens. To understand genetic diversification at these higher taxonomic levels one must include the bacteria ecological context, which is too often unknown for bacterial facultative or opportunistic pathogens. We have queried metagenomic data using the population genomic information on *Acinetobacter* to identify natural reservoirs, characterise niche diversity, and study its evolution in the genus. *Acinetobacter* species can be found in a wide diversity of ecosystems, many of which previously unknown, and these patterns tend to be more similar for closely related taxa. Yet, the change of these patterns with time is not homogenous, showing some ancestral discontinuities that suggest a split of the genus in a small number of ecologically differentiated groups of species.

We have compared the differences of gene repertoires of the genomes at the light of the environmental patterns, and found strongly associated functional traits. In particular, our data shows that *A. baumannii* and its closely related species are often found in human-associated ecosystems, mainly skin and oral mucosas. This association is supported by the specific gene functions that differentiate this large clade from the others, which over-represents functions involved in interaction with the host. Many of these traits were acquired before the massive use of antibiotics by humans, but may have provided a favourable genetic background in an appropriate ecological environment for future emergence as opportunistic pathogens.

Hence, our work sets a basis to understand the role of the ecological constraints in the process of species diversification of bacterial pathogens.

P FMC 9

Combination of DNA stable isotope probing with cultivation-dependent methods for the comprehensive evaluation of the biodegradation potential of *cis*-1,2-dichloroethene.

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The accumulation of toxic lower chlorinated ethenes in the environment is of current public concern. Despite extensive research, *cis*-1,2-dichloroethene (cDCE) degradation has been demonstrated only in few microorganisms, or few mixed microbial cultures. Efficient cDCE mineralization is mostly attainable by means of aerobic cometabolism in the presence of a suitable cell's growth-supporting substrate. However, the molecular mechanisms of such a process are unclear.
The overall objective of this study was to evaluate the cDCE bioremediation potential of microbial communities occurring at an environmental contaminated site. Specific objectives were: (i) establishment of a DNA-based stable isotope probing (SIP) experiment to track cDCE-utilizing microorganisms within contaminated soil; (ii) development of enrichment cultures of soil microbial communities and assessment of cDCE removal by aerobic cometabolism; (iii) isolation of aerobic microorganisms able to cometabolically degrade cDCE, from both contaminated soil and groundwater samples.
Preliminary results confirmed the feasibility of DNA-SIP with ^{13}C-cDCE, since $^{13}CO_2$ evolved over the incubation time course. Prominent cDCE removal, over a period of 10 days, occurred in enrichment cultures in the presence of toluene (82.8%) and phenol (57.8%), while an isolate affiliated to *Acinetobacter* spp. was able to degrade 80.7% of initial cDCE in the presence of phenol.
To the best of authors' knowledge, this is the first time that ^{13}C-cDCE is used for direct analysis of cDCE-derived carbon assimilation by SIP. Downstream analyses of the SIP- and enrichment- communities' metagenomes will elucidate the microbial ecology of the cDCE-contaminated site.
Acknowledgement: The support of Czech Science Foundation is acknowledged (project no. 14-32432S).

P FMC 10

Biosynthesis of microcystin in *Fischerella* sp. strain CENA161: Identification of the *mcy* gene cluster and toxin variants

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Freshwater quality control for recreation and supply is an essential service to the safety and well-being of human communities. Cyanobacteria are potential toxin producers in aquatic environments, and as such their presence must be accurately monitored. Microcystin is a hepatotoxic heptapeptide produced by a non-ribosomal route and is toxic for humans and other animals, and the ecological role of this toxin is still not elucidated. The *Fischerella* sp. strain CENA161 was collected from a spring water in Southern Brazil and produces microcystin. High-throughput sequencing was carried out for elucidation of the microcystin synthetase gene cluster, as well as chemical analyses to identify the microcystin variants produced by the strain. It has been cultivated under controlled conditions for irradiance and temperature, and its genomic DNA was extracted and sequenced on Illumina MiSeq platform using the 600 cycle MiSeq Reagent Kit v3. The chemical analyses were performed by high performance liquid chromatography coupled to mass spectrometry in Triple Quadrupole and Ion Trap, according the molecules fragmentation pattern. The results showed the 10 microcystin biosynthesis genes organized in two operons, a smaller operon formed by *mcyA, mcyB, mcyC* and *mcyJ* genes and a larger operon with the *mcyD, mcyE, mcyG, mcyF, mcyI* and *mcyH* genes. The nucleotide sequences revealed high identity with *mcy* genes from the *Fischerella* sp. strain PCC 9339, although the gene cluster structure is similar to that found in *Nostoc* sp. strain 152. The chemical analysis revealed the production of five known microcystin variants (MC-LR, MC-FR, MC-LA, MC-LM and MC-LL) and two rarely-described microcystin variants (MC-LAba and [Asp^3] MC-LL) evidencing that the gene cluster is active under culture conditions. These results contribute to understanding of the evolution and distribution of microcystin biosynthesis in cyanobacteria, as well as waters quality monitoring and ecological significance.

P FMC 11

Ecosystem structure and potential functional diversity in microbiota-adapted lignocellulosic biomass

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Introduction. Exploration of lignocellulose-adapted microbial communities during the biodegradation of biomass can improve the knowledge in biomass-to-biofuels conversion technology.
Objectives. The aim of this study was to investigate the microbial ecology, taxonomic diversity and dynamic of adapted microbiota of lignocellulose and to explore their potential as source of enzymes for the application in bioenergetics.
Methods. Chipped wood from *Populus nigra* was subjected to natural biodegradation in open field and underwood conditions. Total DNA was extracted from microbes adherent to the plant after 0, 45, 90 and 135 days and used for pyrosequencing on a GS Junior platform. Data were analysed by QIIME and TMeV software to identify species involved in the plant degradation and determine their dynamics. Moreover, DNA sample extracted from *P. nigra* after 135 days underwood bio-deterioration was also sequenced in Illumina platform and data assembled by SOAPdenovo were used to predict open reading frames (ORFs) by MetaGeneMark software.
Results. The lignin-adapted microbiota included several members related to organisms previously characterized as biomass degraders, while others were less well-characterized taxa. Although abundance and trends were affected by time and degradation condition, the predominant families were *Microbacteriaceae*, *Flavobacteriaceae*, *Flexibacteriaceae*, *Sphingobacteriaceae*, *Bacillaceae*, *Rhizobiaceae*, *Sphingomonadaceae* and uncultured bacterium CH21. Analyzing the microbial diversity at a deeper taxonomic level, 60 genera were recovered (incidence ≥ 0.5% in at least two samples). However, bacterial community profiles revealed that the degradation condition determined a selective pressure on the abundance and diversity of taxa retrieved during the process. The Illumina-based sequencing revealed various genes that could be involved in lignocellulose degradation with more than 1500 ORFs related to the carbohydrate metabolism and a prevalence of genes matching glycoside hydrolases and carbohydrate-binding modules.
Conclusion. Sequencing technologies highlight that there are unexplored taxa with important roles in lignocellulose deconstruction and allowed the discovery of natural lignocellulose-degrading microbes and genes that can provide new perspectives for biomass bioconversion.
This work was supported by Industrial research project BioPoliS PON03PE_00107_1.

Keywords: High-throughput sequencing, lignocelulosic biomass, microbiota, glycoside hydrolases, bioenergy.

P FMC 12

Transcriptional profile of *Corynebacterium pseudotuberculosis* 258 biovar *equi* submitted to conditions stress

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The *Corynebacterium pseudotuberculosis* is a Gram-positive pathogenic bacteria, intracellular optional, non-sporulating, non-encapsulated, and pleomorphic . Genome has a 52.2% G + C content and belongs to the class of Actiniobactéria. It is responsible for expressing various diseases, including ulcerative lymphangitis in horses, but can infect other hosts, including humans. The mechanisms related to virulence and pathogenicity factors associated with the spread and persistence of *C. pseudotuberculosis* in the host are poorly understood. Thus, the present study investigated the transcriptional profile of *C. pesudotuberculosis* (biovar *equi*) by simulating the growth conditions in acid stress, thermal and osmotic, found in the host during the infection process. The RNA-Seq libraries of transcripts present in the three stress conditions and control were sequenced in the SOLiD ™ V3 plus platform. The transcripts were aligned against the reference genome of *C. pseudotuberculosis* 258 through TopHat program and the results of differential expression were obtained by Cufflinks program. Differentially expressed were considered, the genes that showed the fold-change ≥ 2 value (induced) or ≤ 0.5 (repressed). The differentially expressed transcripts were functionally annotated by Blast2GO tool, in order to identify the most significant biological processes in the three stress conditions. Analyses showed 500 induced genes differentially expressed and considered. Virulence related genes have been identified, including genes present in pathogenicity islands, such as the *ppsA* gene coding lipid associated with the cell wall was induced thermal stress condition. Hypothetical proteins showed high value of fold change and need to be evaluated in future studies because they demonstrate involvement in bacteria maintenance in the media. The results obtained in this study allowed the identification of a set of candidate genes

studies aimed at developing vaccines or discovery of effective drugs against ulcerative lymphangitis and the caseosoa lymphadenitis.

P FMC 14

Draft genome of the nitrogen-fixing cyanobacteria *Nostoc sp.* CENA 21, isolated from soil sample nearby Solimões river at Amazon, Brazil

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In the current context of biotechnology, cyanobacteria continue to gain attention due to its ability to produce a vast range of natural products of industrial significance. Not only the phototrophic bacteria itself is interesting, but the cyanobacteria-heterotrophic bacteria consortia is very complex in terms of taxonomy, evolution, genes transfer, chemical and ecological interactions. Due to the complexity of these consortia, even after the advance of the next generation sequencing technologies (NGS), offering a massive generation of complete genomes, cyanobacteria genomes remain a challenge for the field. This study aims to reduce the amount of bacterial contamination at the microbiological and bioinformatics level in order to generate a cyanobacteria draft genome suitable to genome mining of new natural products. The organism of study is *Nostoc sp.* CENA 21, an important nitrogen-fixing cyanobacterium, isolated from "várzea" floodplain sediments along the mainstream of Solimões/Amazon River channel. We applied SDS and lyophilization treatments to reduce the number of heterotrophic contaminants attached to the mucilage membrane of the cyanobacteria in culture, before proceeding to the DNA extraction. We sequenced on the Ion Torrent PGM™ and we used bioinformatics tools like BLAST, DarkHorse and QUAST to remove the contigs belonging to bacterial contaminants. The final draft of the *Nostoc sp.* CENA 21 genome consists of 22 contigs with N50 of 654,081, total size of 7.13 Mb and G+C content of 40.56%. The automatic annotation by RAST annotation server identified several genes involved with iron, nitrogen, sulfur and phosphorus metabolism. Other genes found indicate resistance to antibiotics and toxic compounds, like mercury, arsenic and chromium compounds resistance, copper tolerance and resistance to fluoroquinolones and beta-lactamases. The prediction of secondary metabolites using antiSMASH tool pointed 14 biosynthetic gene clusters, including 4 bacteriocins, a NRPS, 5 hybrid NRPS-PKS products, a terpene, a lantipeptide and others. Further detailed analysis of those biosynthetic clusters and comparative genomics are in process and the results will be included in our future publications.

Support: FAPESPA

P FMC 15

Aqueous two phase extraction of *Jonesia denitrificans* xylanase 6 in PEG 1000/phosphate system.

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The impetus for research in the field of bioseparation has been sparked by the difficulty and complexity in the downstream processing of biological products. Indeed, 50% to 90% of the production cost for a typical biological products resides in the purification strategy. There is a need for efficient and economical large scale bioseparation techniques which will achieve high purity and high recovery, while maintaining the biological activity of the molecule. One such purification technique which meets these criteria involves the partitioning of biomolecules between two immiscible phases in an aqueous system (ATPS). The Production of xylanases is carried out in 500ml of a liquid medium based on birchwood xylan. In each ATPS, PEG 1000 is added to a mixture consisting of dipotassium phosphate, sodium chloride and the culture medium inoculated with the strain *Jonesia denitrificans*, the mixture was adjusted to different pH. The concentration of PEG 1000 was varied : 8 to 16 % and the NaCl percentages are also varied from 2 to 4% while maintaining the other parameters constant. The results showed that the best ATPS for purification of xylanases is composed of PEG 1000 at 8.33%, 13.14 % of K_2HPO_4 , 1.62% NaCl at pH 7. We obtained a yield of 96.62 %, a partition coefficient of 86.66 and a purification factor of 2.9. The zymogram showed that the activity is mainly detected in the top phase.

P FMC 16

Linking alpha-diversity estimates between community fingerprinting and high-throughput 16S rRNA gene amplicon sequencing

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Question: Microbial diversity screening by means of high throughput 16S rRNA gene sequencing has become a gold standard procedure for examining changes in community composition and structure. However, a large amount of data on α- and β-diversity has been produced through community fingerprinting methods or 16S clone libraries. Recent studies have shown that β-diversity measured by community fingerprinting is comparable to the estimates through high-throughput 16S rRNA gene sequencing. However, no link is established between the α-diversity estimates of the two methodological categories yet.
Methods: Here, we analyze a large set of data generated by high-throughput 16S rRNA gene sequencing from a variety of habitats, for which community fingerprinting, i.e. denaturant gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) and clone library profiles are also available. We have used publicly available as well as experimentally- and in-silico-generated data accounting for more than 20 different habitats.
Results: We observed a significant, positive correlation between richness estimates deduced from community fingerprinting methods and evenness estimates from high throughput 16S rRNA gene sequencing.
Conclusions: Our results suggest that these two components of α-diversity are directly comparable. That enables the use of evenness estimates from a vast number of past studies for comparison to upcoming studies, as well as the use of community fingerprinting for accurate estimations of evenness. Our results also highlight the need for re-assessment of past studies that were based on richness estimates of low-resolution community fingerpinting methods.

P FMC 17

Predator-Prey Networks in Wastewater Treatment Plants: A Potential Way to Improve Pathogen Removal

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Question: The reuse of treated wastewater is of particular interest in arid regions like the Middle East. However, the necessary removal of pathogenic bacteria is still a major problem in the conventional wastewater treatment processes. An approach to overcome this issue might be the exploitation of predator-prey interactions to remove pathogenic bacteria in waste water more effectively.
Methods: In this trilateral study we investigated the diversity of bacteria and their micropredators, i.e. protists, bacteriophages, and specific predatory bacteria (*Bdellovibrio*-and-like organisms, BALOs) in wastewater treatment plants (WWTP). Wastewater samples were taken monthly over a period of a year from three different treatment plants located in Germany, Israel, and Palestine to track seasonal community changes. We used the Illumina Miseq amplicon platform to analyse the microbial diversity (16S and 18S rRNA genes) of all bacteria, protists and BALOs. In addition, we quantified the target organisms using qPCR. At the same time we recorded chemical and environmental factors which enable us to correlate our findings.
Results: The results show networks of co-occurring microbes connected by mainly positive interactions between the studied micropredators and their prey under different environmental conditions. Functional core sets of bacteria and micropredators were observed. Furthermore, due to our monthly sampling approach we were able to study the dynamics of these networks.
Conclusion: In this study we thus show that understanding the co-occurrence pattern of predator and prey organisms in WWTP can help unravelling the ecological functions of certain species and their potential interactions in the system. We believe that this knowledge can lead to improved pathogen reduction rates and might have implications in future plant management strategies.

P FMC 18

***Staphylocoagulase*, an exploitable intra- and inter-specific public good**

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Staphylococcus aureus and *Pseudomonas aeruginosa* are a major cause of community-acquired infections and one of the leading causes of nosocomial infections. The ability of these pathogens to colonize their hosts depends upon the cooperative production of extracellular factors. Previous studies have shown that *S. aureus* has the ability to "hijack" the host's coagulation cascade by producing the prothormbin activating protein, *staphylocoagulase* (*coa*). Given that this phenotype is important during infections, yet subjected to exploitation, we postulated that *coa* is a "public good", a molecule that provides benefit on a both intra- and inter-species level. We used an experimental evolution approach by culturing these pathogens together in a clinically relevant *in vitro* wound model. Our experiments were started using three bacterial strains: a wild-type *S. aureus* that produces *coa*, a *coa* mutant, and a wild-type *P. aeruginosa*. The relative fitness of each strain was monitored while propagating the bacterial populations through several rounds of culturing. We observed that: (i) *coa* is vital in developing a bacterium-derived matrix; (ii) there are fitness costs associated with *coa* production; and (iii) it is a trait that is exploitable on a both inter- and intra-species level. In agreement with our prediction, we observed that *P. aeruginosa* and *coa* mutant displayed enhanced antibiotic tolerance when present with wild-type *S. aureus* in comparison to that in a monoculture. Our results provide explanations to (1) how such cooperative behaviors can affect the population dynamics and (2) why *coa* negative *S. aureus* are often isolated from clinical infections

Oral presentations

O GCEH 1

Dissecting massive methanotrophic biofilm formation in a mineral spring cavern

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Caves are known to host a rich diversity of microorganisms often organized in microbial biofilms. Here, we investigate massive microbial biofilms recently discovered in the cavern of a former medicinal spring in southern Germany, where iodine-rich reduced formation water reaches the surface. The biomass produced by microbes and exopolysaccharide slime completely covers the walls and ceilings of the cave, the latter bearing snottites of up to 10 cm length. However, the nature of these unique biofilms as well as their ecophysiology is not yet understood. We hypothesize that methane emerging with the formation water is a major driver of biofilm formation, possibly also involving a coupling of methane and halide cycling.
We sampled microbial communities from horizontal and vertical transects in the cave employing in-depth 454 pyrotag sequencing thereby identifying bacterial taxa and estimating biofilm diversity. Gas emissions and the cavern atmosphere were characterized by GC-IRMS analyses. The assimilation of methane-C into biofilms was evaluated by stable isotope analysis. Potential methane oxidation rates were evaluated in biofilm incubations. Methane concentrations were high within the spring water (up to 50 %) but at an average of 0.5 % in the cavern atmosphere. Stable isotope analysis confirmed the fossil origin of the methane (-41 ‰ $\delta^{13}C$; -165 ‰ $\delta^{2}H$).
Our primary taxonomic characterization of the biofilms revealed a surprisingly diverse polymicrobial community assembly, with *Alpha-* and *Gammaproteobacteria* dominating the biofilms. Discrete patterns in community assembly and biochemical composition of biofilms were found, indicating local selection of biofilms by environmental gradients. Distinct methanotrophic and methylotrophic populations within the *Methylophilaceae* and *Methylococcaceae* were abundant on the walls and especially in the cavern water, but not so in ceiling biofilms. Here ribosomal and functional gene analysis hinted at methylotrophic and potentially halide cycling populations, supporting a postulated production and oxidation of methyl iodide in this unique habitat. In conclusion, primary insights into a dominantly chemolithoautotrophic natural biofilm system are revealed, thriving on deep subsurface energy inputs but only a few meters under our feet.

O GCEH 2

Effects of alternative water events on soil microbial communities from contrasting aridity zones of the Namib Desert

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Arid systems constitute the most extensive and one of the harshest terrestrial biomes on Earth. In these ecosystems, microbial metabolism is strongly affected by water availability, including the timing, intensity and frequency of water events.A field study along a naturally occurring aridity gradient across the Namib Desert was performed, where soil were sampled every 10 km along a 190 km transect. Desert soil bacterial community structures (16S rRNA gene TRFLPs) and function (extracellular enzyme activities) were found to change drastically with increasing xeric stress. Based on the field study, one soil from the most hyperarid region and one from a less arid zone, were used in a controlled mesocosms study. The aim of this controlled study was to assess the response of microbial communities from soil presenting different water-regime histories when pulsed with wetting events of different frequencies and intensities related to modelled climate changes scenari. A total of 18 mesocosms (23 dm^3) were placed *in situ* in the Namib Desert. During 32 days we simulated either actual or predicted Namib Desert precipitation regimes. The diversity of bacterial and archeal (16S) as well as fungal (ITS) communities was assessed by high through-put sequencing in a total of 80 samples.These studies clearly demonstrate the complexity of microbial community responses to altered water availability in hyperarid ecosystems and underline the high dynamism of hot desert microbial communities.

O GCEH 3

Growth of *Geobacter metallireducens* under environmental conditions with carbon limitation in sediment columns indicates new types of regulation of catabolic pathways

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Despite many years of studying gene regulation under laboratory conditions almost nothing is known about gene expression of microorganisms in their natural habitat. Fundamental chemostat studies provided strong indications that concepts of gene regulation like diauxy are probably laboratory phenomena induced by artificially high substrate concentrations in batch cultures.
Here, we examined the physiology of the anaerobic, iron and nitrate-reducing delta proteobacterium *Geobacter metallireducens* along a substrate gradient in sediment columns packed with natural or artificial quartz sand. Benzoate was used as carbon and electron source with nitrate as electron acceptor. Changes in gene expression were examined by a proteomic approach using nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS) with subsequent label-free quantification. Despite benzoate was the only provided substrate, *G. metallireducens* expressed the toluene degradation pathway together with phenol- and *p*-cresol-degrading proteins at the interface between high (0.5-1 mM) and low concentrations (below 100 μM). Furthermore, genes for ethanol and butyrate degradation were highly expressed at benzoate concentrations below 80 μM indicating an adaptation to utilization of alternative electron donors rather than benzoate only. The data indicate that in sediments *G. metallireducens* is prepared to degrade aromatic hydrocarbons or alcohols and short chain fatty acids although the respective substrates are not present. We conclude that under environmental conditions and being sessile on the sediment, *G. metallireducens* adopts a type of gene regulation that is similar to the de-repression of catabolic genes observed for *E.coli* in chemostats. This leads to a baseline expression of all catabolic genes even in the absence of inducers and is supposed to enable organisms to consume several substrates simultaneously. Moreover, we show here that under environmental conditions some organisms like *Geobacter metallireducens* express a subset of distinct catabolic pathways ("a standard program") which allows them to simultaneously consume substrates typically occurring in the habitat.

O GCEH 4

The effect of adhesion forces between bacteria and anode on electron transfer in microbial fuel cells

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Microbial fuel cells (MFCs) have been recognized as a potentially promising biotechnology that converts organic matter into electricity using bacterial biofilms as biocatalysts. Currently, one of the main process restrictions is the slow kinetics of electron transfer from bacteria to the anodic electrode surface. This anodic electron transfer is based on the necessity for some bacteria to find an electron acceptor for the electrons liberated during oxidative substrate degradation. Most of current-generating microorganisms are metal reducing bacteria that have developed adaptation strategies to transfer their electrons to insoluble electron acceptors such as iron or manganese oxides. The resistance to electron transfer from bacterial membrane to the anode probably plays a major role in this activity. So the objective here was to study the effect of the bacterial adhesion force and the effect of electrostatic forces on anodic respiration of an electroactive bacterial model such as *Shewanella* or *Geobacter*. We hypothesized that increased adhesive forces would increase electron transfer kinetics. First, the interaction strengths were evaluated. The interaction energy was calculated with the extended DLVO theory including electrostatic forces, and the adhesion strength was measured experimentally in parallel-plate flow chambers for different anodic electric potential. Then, the MFCs were operated with electroactive bacteria and with acetate as the substrate. Different anodic electric potentials were applied. Acetate consumption was measured by liquid chromatography (HPLC). Electron transfer kinetics were calculated based on measured electrical current and voltage. Finally, the intracellular NADH concentrations were measured by enzymatic dosage. These results will help determine the effect of engineering parameters on the bacteria/anode interaction, and the effect of electrical potential on anodic respiration activity in order to improve the MFC performance

Poster Presentations

P GCEH 1

Diet determines bacterial diversity and community structure in Bornean pitcher plants

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Carnivorous plants of the genus *Nepenthes* have been studied for over a century, but surprisingly little is known about associations with microorganisms [1]. The two species *Nepenthes rafflesiana* and *Nepenthes hemsleyana* differ in their primary nutrient source, sequestering nitrogen from arthropod prey and bat faeces, respectively [2]. We expected bacterial communities living in the pitchers to resemble this diet difference. Samples were taken from different parts of the pitchers (leaf, peristome, inside, outside, digestive fluid) of both species. Bacterial communities were determined using culture-independent high-throughput amplicon sequencing. Bacterial diversity and community structure was comparable across plant species and most tissues, except digestive fluids. These showed opposing trends with *N. hemsleyana* harbouring a more diverse bacterial community. In *N. rafflesiana* fluid, high levels of *Acidocella spp.* implied a close association with the plant. In *N. hemsleyana* fluid, vertebrate gut symbionts as well as saprophytic taxa could be detected, the latter of which might act as competitors for nutrients. Generally, digestive fluid communities were highly variable in structure, which might be applicable to a difference in digestion status. Nitrogen-fixing bacteria were present in both study species, which might provide essential nutrients to the plant at times of low prey capture and rare encounters with bats, respectively.

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P GCEH 2

Biogeography of the western Swiss Alps: teasing apart the effect of the environment on the soil bacterial communities.

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The possible effects of climate change on biodiversity is of major concern for scientists, policy-makers, and laypeople. The wide elevational gradients and topographical heterogeneity in the Alps present a unique opportunity to study the effects of climate and land-use changes on this biodiversity. Indeed, the mean annual temperatures in the Swiss Alps have increased by 0.57ºC per decade, while the northern hemisphere has increased by 0.25ºC per decade. Over the last decade, an ongoing project at the University of Lausanne has extensively investigated plant-plant and plant-insect interactions within a 700 km2 area of the Western Swiss Alps, and used these data to model and predict niche-based migration patterns in a possible future with a changed climate. However, despite the exhaustive scientific resources available for macroorganisms and abiotic processes, there is currently no systematic data available on the microbial diversity associated with the study sites. In order to fill this knowledge gap and to allow us to study the alpine biodiversity from a more holistic perspective, we have begun to investigate the bacterial community diversity in the alpine top-soils across an elevational gradient of 500-3000 m at the same sites where plant and insect data have been previously collected. Here we will present first results of soil bacterial community analysis across more than 100 sites and the correlations of soil bacteria occurrence with environmental data and plant species distributions.

P GCEH 3

Gradient of soil organic matter quality affects microbial community function and activity in forest soils

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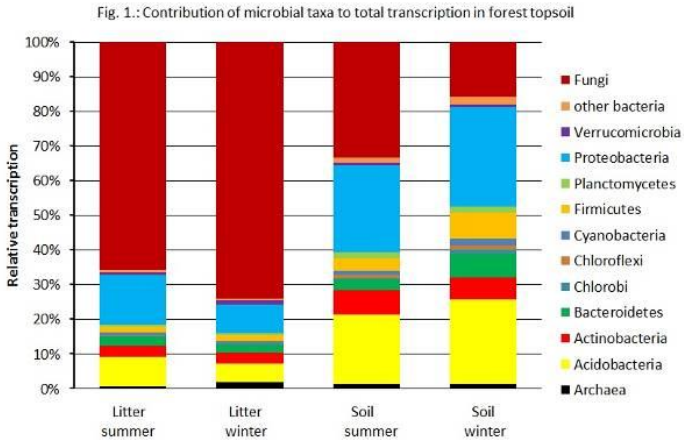
In acidic temperate forests with little invertebrate mixing, accumulation of root litter on the forest floor and its decomposition leads to the development of the vertical gradient of soil properties including organic matter and nutrient content and microbial biomass. While the differences in microbial community composition among soil horizons are well known, the distribution of microbial taxa across the depth profile at a finer scale and the differences of microbial activity along this gradient remained unexplored. The aims of this work was to thus to describe the composition of soil bacterial and fungal communities across the vertical gradient of forest soil at a fine spatial resolution and to combine metatranscriptomics, microbial community analysis and enzyme analyses to describe the role of individual microbial taxa in the functioning of the forest topsoil across such gradient. The results show that the microbial communities along the soil vertical profile are highly diverse and exhibit high spatial variation even within the existing horizons. This applies for both the community composition and function, analysed as the transcript pools. Fungal contribution to total transcription decreases with soil depth representing >60% in the litter compared to 16-33% in the soil (Fig. 1). In addition, the functional profiles of expressed genes across all microbial domains also change. Importantly, the relative expression of hydrolytic enzymes involved in organic matter decomposition also decreases with soil depth while the expression of microorganisms associated to plant roots increases, especially in summer. Our results indicate that organic matter decomposition and rhizodeposition are both important drivers of the differences across the soil depth profile. The importance of the latter process changes with the change of plant photosynthetic activity and indicates an important feedback among the plant and microbial activity.

Figure 1



P GCEH 4

Plastic debris: a distinct niche in the marine environment

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Living the plastic age has major implications on the marine environment, from entanglement of birds, fish and mammals to ingestion of plastic debris.
Recent research shows that marine plastic litter (MPL) is colonised by micro-organisms, especially bacteria (Zettler et al., 2013). These bacteria can be alien or invasive, using the plastic as a transport vector. Some of these bacteria could be pathogenic for man and animals, while other could be beneficial and for instance biodegrade the plastic particles.
In this research we aim at identifying the major bacterial taxa present on MPL. To investigate the source of bacteria living on marine plastics, we conduct a comparative analysis against bacterial communities of the nearby environment (sediment, seawater). Parameters influencing the plastic bacterial composition were investigated as well.
Samples were taken at multiple locations along the Belgian part of the North Sea during several seasons. 16S (V3-V4) amplicon sequencing was used to study their bacterial communities.
Differences in bacterial community structure and diversity showed that plastic represent a distinct microbial niche compared to sediment and seawater. Most of the bacterial families on plastic were identified in sediment and/or seawater. However some bacterial families identified on plastics, e.g. Vibrionaceae and Pseudoalteromonadaceae, are rarely found in sediment and seawater. The bacterial diversity of plastic was higher compared to seawater and in the same range as sediment. In addition, a high diversity was observed between bacterial communities of the plastic samples, which could not always be related to differences in sampling location or date. Apart from environmental parameters like salinity, pollution and sampling depth, that may influence the plastic bacterial communities, less obvious factors such as chemical adsorption, additives of the plastic and biofilm formation stage might contribute to bacterial colonisation patterns on marine plastics.
Based on these observations MPL was defined as a distinct microbial niche in the marine environment, influenced by environmental and plastic-related factors.
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P GCEH 5

Microbial community structure and function vertical distribution in snowpack over sea ice from Greenlandic fjord

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Seasonal snowpack can extend over 14% of the total Earth surface at times, covering up to 46 million km^2 of land and 25 million km2 of sea ice. Far from sterile, snow has recently been shown to have an unexpected abundance and diversity of microorganisms with average of 10^3 and up to 10^5 cells per mL of melted snow and with representatives of numerous microbial taxa among Bacteria, Archaea and Eukarya. Although these microorganisms have been detected, the ecology of the snowpack microbial habitat remains largely unknown, especial the selection of specific microbial communities by variable environmental conditions and the resulting functional signature reflecting the snowpack environment. In this study, we focused on snow over sea ice with samples collected in the vicinity of Nuuk in South West Greenland in a fjord with snow covered sea ice. A vertical gradient: seawater, sea-ice, snow and atmosphere, was sampled. Within the snow, four layers identified by visual structure, a first thin hard top layer in direct contact with the atmosphere, one basal saline snow wetted by brine ascendant flow and two intermediate layers, were sampled. We addressed how the microbial communities are influenced by their different seeding sources, atmosphere and sea-ice, and what were the specific functions involved in response to abiotic characteristic of the snowpack (*e.g.* UV-light exposure and salinity). We applied a combined metagenomic and metatranscriptomic approach to each component of this vertical gradient sea water, sea-ice, snow and atmosphere. Comparison of distribution of sequences in taxonomical and functional groups among all samples helped identify common and specific patterns of microbial communities in this complex habitat. The expression pattern induced in part from the RNA sequencing could lead to a better understanding of which microorganisms are the most active, what their dominant metabolic processes are, and how rapidly they respond to this highly fluctuating environment.

P GCEH 6

Halophilic Bacteria and Archaea of Internal Saltmarsh in Saudi Arabia

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Such extreme environments like Continental or Inland Sabkha are formed when the surface is in parallel and near to groundwater storages, where the upper soil's particles are removed continually and groundwater raise to subsurface. High temperature and seasonal rainfall increase the rate of water evaporation leaving salts to be accumulated at the upper 50 cm. This kind of habitat with extraordinary concentration of different types of salts defines its bacterial community as halophiles. This work aimed to identify the species down to genus level at the present of physicochemical properties within this ecology. A core sample of depth of 80 cm was collected from inland saltmarsh called AlShiggah in AlQassim District, Saudi Arabia, which is geologically considered as a part of continental or inland sabkha. Then, the core was cut horizontally into six layers (samples) at different intervals as following; I = 3-5 cm, II = 10-15 cm, III = 15-20 cm, IV= 20-30 cm, V = 35-60 and VI = 60-80 cm. Anions and cations (sulfate, nitrate, chloride, sodium, potassium, calcium, magnesium) were determined by appropriate analytical methods and instruments based the targeted analytes while total iron and manganese were measured by ICPMS. Diversity assay of bacterial communities was performed by 454 pyrosequencing for 3k reads. Overall, samples (I-VI) are considered salty, sulphurous and strong saline > 0.12 ms/cm with ratio of 5:1. Chloride and sodium were at the range of 299,912-573,337 and 175,000-350,000 ppm, respectively for all samples except sample I (3-5 cm). Nitrate wasn't detected while sulfate varies between 24,750 to 57,812 ppm for all samples. The average concentration for total iron and manganese, which are utilised as source of energy by microbes, were 11,350 and 235 ppm, respectively. Cross the samples, bioinformatics analysis showed that bacteria was dominated by 86 % while archaea existed with 14%. Most of genera detected were belong to *Cyanobacteria, Actinobacteria*, Bacteroidetes, *Halobacteriaceae* (Haloarchea). In addition, Sulfate-reducing bacteria (SRB) like *Desulfobacterium* spp., *Desulfobacca* spp. and *Desulfotomaculum* spp. were detected. Such an area like AlShiggah, as internal saltmarsh, might keep a promising niche for potential microbes of which can be utilised further for industrial applications.

P GCEH 7

A microfluidic chip to measure bacterial chemotaxis

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Chemotaxis is a behavior by motile bacteria to sense the environment and swim in the direction of or away from chemical compounds. In a uniform environment bacteria swim randomly to explore the maximum space but when they are in presence of a gradient of attractant, they bias their swimming direction toward the highest concentration of attractant. Chemotaxis is rapid, and could thus be exploitable for developing biosensors with quick response. In addition it is conserved among motile bacteria and some species show chemotaxis toward toxic compounds. In order to measure chemotaxis quantitatively at the minute scale, we used microfluidic chips.

Here we pursue the design of microfluidic chips in which a gradient of attractant can be generated which enables measurement of bacterial chemotaxis. The principle of the design is based on filter channels with a height of 700 nm, which allow the diffusion of small chemical molecules but prevent the passage of the bacterial cells. The chips are composed of an inner channel, where cells are introduced, connected via the filters to two parallel side channels, in which attractant solution and buffer are flowed in order to produce a gradient. The attractant molecules diffuse between the source and the sink channel, which creates a stable gradient perpendicular to the flow in the inner channel.

Motile cells detect the gradient and swim toward the highest concentration of the attractant, which leads to an accumulation of cells on one side of the channel. The chemotaxis response of fluorescent bacteria is observed by microscopy and the distribution of the cells across the channel is determined.We show that chemical gradients can be produced in a microfluidic design consisting of three parallel channels and that *Escherichia coli* cells introduced in the middle channel experience chemo-attraction toward different molecules and concentrations. Moreover, this flow-based microfluidic chips offers the possibility of continuous measurements with flow of different samples and reuse of cells.

P GCEH 9

Bacterial diversity in freshwater polar environments of Svalbard: bioprospecting for novel low temperature active hydrolases

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Introduction: Glaciers represent approximately 10% of the global terrestrial surface and accommodate various low temperature active biota. Svalbard Archipelago has recently attracted much attention in assessing the impact of anthropogenic activities and global warming on the microbial ecology. However, information on the structure and spatial and temporal dynamics of microbial communities remains less researched for the southern area of Spitsbergen.
Objectives: In this study, we combined culture-dependent and -independent approaches to examine the bacterial community distributions in geologically and hydrologically diverse regions of Svalbard, in the vicinity of Hornsund fjord. In addition, all bacterial strains isolated were evaluated in terms of their low temperature active hydrolytic enzyme activities.
Materials & Methods: Bacterial communities were characterized by 16S rRNA gene MiSeq sequencing. Bacterial strains were isolated using aerobic heterotrophic conditions. These isolates were examined for their ability to produce extracellular low temperature hydrolytic enzymes. Bacterial strains with high hydrolytic activities were selected for further investigation.
Results: Bacterial communities consisted mainly of *Bacteroidetes*, *Betaproteobacteria, Planctomycetes* and *Microgenomates* (OP11). A remarkable *Microgenomates* population was identified in certain freshwater samples. Principal component analysis of physicochemical and biological data revealed two main biotic and an abiotic factors. Among the 300 bacterial strains isolated, 9.7%, 43%, 33.5% and 45% exhibited lipolytic, cellulolytic, xylanolytic and proteolytic activity respectively even under low temperature conditions. Only 6% of the isolates were able to hydrolyze all the examined substrates at low temperatures and these stains were selected for further investigation.
Conclusion: Bacterial communities in polar freshwater ecosystems examined mainly consisted of aquatic microbiota involved in detritus mineralization, while zooplankton-associated taxa comprised of a significant part of bacterial communities. In addition, the ability of the newly isolated strains to produce a broad spectrum of low temperature active enzymes is of great interest for both fundamental research and biotechnological applications

P GCEH 10

Adaptation of *Methylocystis* sp. strain SC2 to salt stress revealed by global transcriptome analysis

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Introduction: Salinity has been shown to have a major effect on the composition and activity of methane-oxidizing bacteria (MOB) communities in dryland soil, affecting in particular the relative abundances of types Ia, Ib, and II MOB. However, the effect of salt stress on the genome-wide expression of a particular MOB has not yet been studied.
Objective: Assessment of short-term and long-term effects of salt stress on the transcriptome of the type II methanotroph *Methylocystis* sp. strain SC2.
Material & methods: Strain SC2 was grown to the mid-exponential phase and then treated with 0.75% NaCl. Exposure time to NaCl was 0 min, 45 min, and 14 hrs. Control and NaCl treatments were analyzed in triplicates by Illumina RNA-seq. Transcripts were mapped to the SC2 genome and expression levels were calculated using the CLC Genomics Workbench. Normalized gene expression levels were calculated and reported as RPKM values. Log_2 fold changes in RPKM values of ≤−2 and≥ 2 were considered significant.
Results: A total of 371 (out of 4058) genes encoded by the genome of strain SC2 were identified as differentially expressed in the 45 min and/or 14 hrs treatments. Only a set of 62 genes was differentially expressed in response to both treatments. The number of down-regulated genes decreased from 169 in 45 min to 32 in 14 hrs, indicating that strain SC2 adapted to salt stress with treatment time. The expression level of genes involved in cell membrane and cell wall synthesis, stress response, and transcriptional regulation, but not those involved in methane oxidation, was significantly affected in both NaCl treatments. However, the expression patterns were remarkably different between short-term and long-term responses. Genes that showed high up-regulation only in the 14 hrs treatment include the complete *VirB* operon. The 11 genes of this operon encode the regulatory VirB, which is hypothesized to form a membrane-localized T-DNA transport apparatus and to be required for processing and transmission of specific segments of a plasmid.
Conclusions: Significant changes in the genome-wide expression occurred in response to salt stress, with the exposure time to salt stress as a critical factor. The *VirB* operon is located on plasmid pBSC2-1, suggesting that this plasmid may play a major role in the response of *Methylocystis* sp. strain SC2 to salt stress.

P GCEH 11
Microbial communities in the
CO_2-dominated active fault zone in NW Bohemia
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The Cheb Basin (Czech Republic) is a shallow neogene intracontinental basin filled with fluvial and lacustrine sediments [1]. It is assumed that the rise of magmas and the fluid activity are the forcing mechanisms for seismic activity [2, 3]. Fluid enriched with mantle-derived CO_2 is degassing through conduits to the surface (dry mofettes) or get mixed with groundwater (wet mofettes) [4]. There are hints that the microbial turnover (e.g. CH_4 production) of this region is significantly accelerated after swarm earthquakes [5]. This observation leads to the question whether earthquakes can trigger microbiologically driven processes by delocalization of substrates and environmental changes. To understand these geo-bio interactions and to determine to which extend the microbial communities are conditioned by CO_2 degassing it is necessary to analyze the community structure in detail. Therefore mineral water (well head) as well as fluid from three wet mofettes was sampled. Furthermore, five sediment cores were retrieved from depths of maximal 8 m, covering areas of different CO_2 concentrations. Here we present first insights into the community structure in CO_2 influenced fluids and sediments by applying genetic fingerprint analyses (DGGE), high-throughput DNA sequencing, quantitative PCR and geochemical analysis. In fluids and sediments, chemolithoautotrophic, anaerobic/ microaerophilic microorganisms connected to sulfur (e.g. closest relatives *Sulfurimonas autotrophica and Sulfuricurvum kujiense*) and iron cycling (e.g. closest relatives *Gallionella ferruginea, Geothrix* sp.) were highly abundant. In conclusion the autochthonous communities are well adapted to the elevated CO_2 concentrations. However, in fluids from the mofettes an imprint of the common soil community was observed. The ongoing sequence analyses and a planned 100 m deep drilling will provide a more detailed overview of both, bacterial and archaeal communities and help to understand the complex geo-bio interactions in this extreme environment.

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P GCEH 12
Coping with copper: Soil active bacterial communities following 100 years of copper contamination
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Since the 18th century, copper derived pesticides have been massively used for wood impregnation and plague control in agriculture. However, little is known about their long-term impact on soil microbial communities and their related ecosystem services.
A former wood impregnation site in Hygum, Denmark, was intensively contaminated with copper in the early 20th century. Today, the site represents nearly 100 years of permanent local exposure and offers a remarkable opportunity to shed light on this problem. A very stable and sharp contamination gradient was discovered, ranging from normal soil copper levels to more than 4 g Cu kg^{-1}.
Our aim was to assess the structure of the potentially active soil bacterial communities along the copper gradient over the course of an entire year. For that purpose, we established an integrated approach combining metagenomics and metatranscriptomics, followed by amplicon sequencing of 16S rRNA gene transcripts.
Three areas of 16 m^2 located along the copper contamination gradient were selected, corresponding to a control (≈15 mg Cu kg^{-1}), a semi-contaminated (≈500 mg Cu kg^{-1}), and a hot spot area (≈4 g Cu kg^{-1}), and sampled every 3 months over the course of a year.
The results indicate extreme differences in the life-style of bacteria in the three different areas, the copper being the main driving force. Thus, with increasing copper concentration we observed a decrease in richness, diversity and bacterial taxa with known importance in the C and N cycles as well as an increase in Nitrospira. Seasonal fluctuations were detected but were significantly reduced in the hot spot, which is in agreement with its low bacterial richness and corresponding lack of ability to respond to variation in e.g. soil temperature and moisture.

Our data contribute with key elements for understanding evolution of soil quality after long-term exposure to recalcitrant pesticides and how this perturbation may impact soil ecosystem services and functionality.

P GCEH 13

Metagenomic analysis of microbial community structure of the acid mine drainage from Transbaikal Area, Russia

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Acidic sulfur-rich wastewaters are the by-products of a variety of industrial operations such as galvanic processing and the scrubbing of flue gases at power plants (Johnson, 2000). The major producer of such effluents is, however, the mining industry. Waters draining active and, in particular, abandoned mines and mine wastes are often net acidic (sometimes extremely so). Such waters typically pose an additional risk to the environment by the fact that they often contain elevated concentrations of metals (iron, aluminum and manganese, and possibly other heavy metals) and metalloids (of which arsenic is generally of greatest concern).
We analyzed the microbial community of acidic water (pH 2.65; T +6.5°C) from the abandoned borehole near an open pit of the tin mining site at Sherlovaya Gora in Transbaikal Area, Russia. We used for characterization of microbial communities the pyrosequencing of the total community DNA.
Metagenomic DNA sequencing revealed that dominant group in this community is *Betaproteobacteria* (90%). In this phylum prevailing organism was bacteria phylogenetically close to the genus *Gallionella*, known to comprise aerobic Fe-oxidizers. In addition to this dominant species we identified bacteria related to the genera *Acidithiobacillus*, *Acidiphilium*, and *Acidisphaera*. Metagenomic analysis showed the presence of pathways for CO_2-fixation, iron oxidation, and nitrogen fixation. Overall, the results of this study provide new information regarding previously uncharacterized environment and show importance of high-throughput sequencing in the study of complex ecosystems. This work was supported by the Ministry of Education and Science of Russian Federation (project ID RFMEFI57514X0001).

P GCEH 14

Biogeographical distribution of Class I integron as bioindicators for freshwater systems

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Introduction. In ecology, bioindicators are species or group of species, which reflect the quality of an environment. Class I integrons can be considered good bioindicators because: i) they are widespread among commensal and pathogenic bacteria associated with humans and animals, even outside clinical context; ii) this class has been the first class discovered and is the most studied; iii) they are often quickly disseminated among the environmental microbial communities; iv) the genetic structure is conserved and well described.
Aim. To test the potential utilization of Class I integrons as bioindicators for freshwater environments.
Methods: The study was conducted in Zhangye (Gansu Province, China), a city that is quickly growing in importance due to its industrial and agricultural development. Samples from highly polluted channels were collected in four different zones according to anthropogenic influences: urban area, agricultural area, industrial area and natural park area.
Results. Culture-independent analysis on the overall microbial communities showed the absence of Class I integrons in the natural park area. Moreover, we found the predominance of gene cassettes in the areas affected by pollution of animal and human origin (agricultural and urban area) probably due to the use of antibiotics to treat disease in farming and in clinical setting. Differently, a higher occurrence of Class I integrons lacking gene cassettes where detected in the industrial area. There are two possible scenarios that explain this result. The gene cassettes were excised (or a priori missing) in absence of antibiotics selective pressure and Class I integrons did not yet capture appropriate gene cassettes that permit a defense against the respective environmental contaminants. It is also possible that integrons are co-selected for with the genetic region they are associated with. In clinical samples, Class I integrons are often associated with plasmids or embedded in Tn21-like transposons that confer resistance to mercury which is one of the pollutants in the industrial sampling site.
Conclusion. Class I integron succeed as effective diagnostic tools to highlight the disturbance due to the different land use.

P GCEH 15

The genetic structure and characterisation of environmentally-associated *Escherichia coli* from water catchments in Eastern Australia.

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Escherichia coli is one of a few universal indicators used in the assessment of faecal contamination in water. It was chosen because it is the most often encountered facultative anaerobe in the mammal gut, it is easy to identify, and typically persists for less than 3 days outside a given host. Therefore the long held belief has been that if *E. coli* is detected in a water body, it is likely to come from a faecal source, has the potential to cause disease in humans, and suggests the possible presence of other enteric pathogens.

Recently, studies show not all *E. coli* cells respond in the same way to the transition from the host to the external environment. It has been demonstrated that specific strains within the B1 phylogroup, have an enhanced persistence greater than 7 days in various external environments, such as water and sediment. Importantly, their presence in water is not the result of recent faecal contamination, but from their ability to exploit the available nutrients. Furthermore, they are not a direct risk to human health as they are susceptible to most antibiotics and lack the required virulence genes for pathogenesis. This study aims to further our understanding of environment-adapted *E. coli* strains from water sources.

Whole genome sequencing was conducted on 97 B1 *E. coli* strains repeatedly isolated from various water catchments across eastern Australia. Their repeated isolation indicates these strains are over-represented in water samples relative to other B1 strains. Antibiotic resistance was found in 8 strains, tetracycline resistant genes were detected in 5 isolates, aminoglycosides in 3, sulphonamides in 3 and trimethoprim resistant genes in 1 isolate. Virulence gene analysis identified 22 genes; *iss* was detected in 52 isolates, *astA* in 10, *cdtB* in 9, *f17G* in 7 and *f17A* in 3 isolates. The remaining 17 virulence genes were detected only once (or twice) in just 5 strains. The lack of virulence genes and particular combinations (e.g. *f17A* and *cdtB*) suggests they are unlikely to cause disease in humans.

This study enhances our understanding of the environmentally-associated *E. coli* strains and has significant implications for its use as a water quality indicator. Where some 'faecal' high cell counts may be over-estimated if these particular strains represent the majority of a water sample.

P GCEH 16

Bacterial diversity of Paraguaçú: a river in Brazilian Semiarid biome

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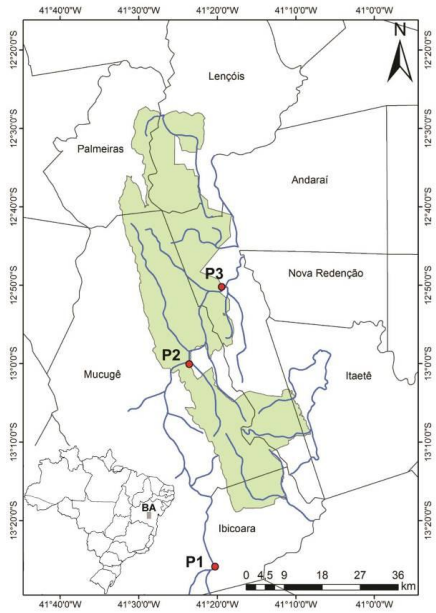
The Caatinga is a Brazilian biome with endemic biota and possess the highest diversity when compared to other biomes with similar climatic conditions. However, the Caatinga is the least studied biome of South America, nonetheless around 50% of Caatinga original area was modified by anthropogenic action. Nearly none study about microbial diversity has been done, especially in aquatic ecosystems in the Caatinga. The aim of the present study was to describe the bacterial community in three points - P1, P2 and P3 - of Paraguaçú River (an important river of Caatinga biome) by means of 16S rRNA gene pyrosequencing and its response to climate variation. In general, the 454 pyrosequencing revealed a pattern of bacterial community similar between all samples analyzed but some differences was annotated. The massive presence of Proteobacteria phylum, in special Betaproteobacteria, was observed in all points and weather conditions studied. The order Burkhoderiales (Betaproteobacteria) was the most abundant, about 70% of total, and it was mostly represented by two families: Comamonadaceae and Oxalobacteraceae. Other classes of Proteobacteria (Alphaproteobacteria, Gammaproteobacteria and Deltaproteobacteria) and the phylum Verrucomicrobia were less abundant. In the principal coordinate analysis (PCA), P1 samples clustered together and separated of other samples because of high ammonia, nitrite, nitrate, silicate and particulate organic carbon (POC) contents. These elevated concentration values might indicate an intense anthropogenic action in this sampled point. The P1 point was the only one located outside of National Park protection. In addition to the factors showed, P1 point presented a higher OD1 phylum and MVS-40 order (Acidobacteria phylum) abundance in wet conditions than other points. In the dry season, P1 point presented higher abundance of ZB3 phylum, Flavobacteriaceae (Bacteroidetes phylum), and Rhodocyclaceae (Betaproteobacteria class) families than other points. These microorganisms are associated to anaerobic environments and to degradation of compounds as sulfur, nitro-

compounds and phosphorus. Potentially these bacteria are involved in the remediation process of this point and could be a great source of genes related to several biotechnological processes.

Figure 1



P GCEH 17

Dynamics of bacterial communities in cryoconite holes of temperate glaciers

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Introduction: Cryconite holes are small depressions of glacier surfaces filled with water and containing sediments on the bottom. They are considered the most biologically active environments on the glaciers. Most of the microbiological studies on these holes focused on polar ice sheets while those of mountain glaciers in temperate and tropical areas remain under investigated.
Aims: The aims of this work were: to compare microbial communities of cryoconite sediment from Forni glacier (Italy) and Baltoro glacier (Pakistan) by describing their taxonomic and functional biodiversity; to investigate the temporal dynamics of microbial communities in cryoconite holes during the ablation season in Forni glacier; to assess the contribution of eolian transport from periglacial environments to the composition of the microbial communities of cryoconite holes.
Methods: A total of 111 samples of cryoconite sediment were collected on the two glaciers in different sampling campaigns; 24 samples of various periglacial materials were also collected on Forni glacier. After DNA extraction, an Illumina sequencing of the V5-V6 hypervariable regions of the 16S rRNA gene was performed. The abundance of bacterial ribosomal operons was quantified with qPCR. Finally, on 12 selected samples whole metagenome sequencing was carried out.
Results and Conclusions: Quantification results indicated the presence of high amounts of bacterial ribosomal operons in cryoconite sediments (10^9-10^{10} copies per gram of sediment). The phylogenetic analysis showed that microbial communities from Baltoro glacier were completely different from those of Forni glacier, being the former richer in heterotrophic bacteria and the latter richer in autotrophic populations. On Forni glacier, the structure of bacterial communities also changed along months during the ablation season, thus suggesting the existence of an ecological succession. Finally, microbial

communities of periglacial materials were very different from those of cryoconite sediments, thus indicating that the latter populations may be selected by the peculiar properties of cryoconite holes rather than simply assembled by transport of material from other sources. Metagenomic analyses allowed to cast a first glance on microbial community functions in this environment.

P GCEH 18

Spatiotemporal patterns and adaptation mechanisms of microbial communities in alkaline soda pans

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Astatic soda pans of the Pannonian steppes are unique environments regarding their physical and chemical characteristics (high turbidity, pH, salinity and special ionic composition), thus they provide extreme habitats for aquatic life. However, compared to other similar habitats worldwide, studies focusing on these lakes are underrepresented in the literature. Our research aimed to obtain a more detailed view about the structure and function of microbial communities inhabiting several soda pans of the Kiskunság National Park (Hungary), combining recent tools of metagenomics and classical methods of microbiology. Results showed a taxonomically and functionally complex microbial community structure. Interestingly, members of the domain Archaea were underrepresented in these extreme habitats. Bacterial composition was remarkably different in the two main types of the pans, the 'turbid-white' and the 'non-turbid-coloured'. A survey for over a year showed that changing environmental conditions (e.g. bird migration, algal blooms and desiccation) have significant effect on the seasonal patterns of the communities. A functional metagenomic approach revealed various strategies of adaptation to these changing environments (genes involved in membrane transport processes, synthesis of compatible solutes and membrane lipids). (This work was supported by the Hungarian Scientific Research Fund, OTKA PD 105407 and PD 112449.)

P GCEH 19

Comparison in two water microbial populations of iron mining region disabled by cultivation techniques and potential of enzymes production.

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In residual water of mining areas there is a huge diversity of microorganisms with many complex biological systems. The activity of them, associated with the degradation of various compounds is related to the minimization of environmental pollution. Biodegradation is accomplished through the use of contaminants, principally as a carbon source, which enables the division of bioremediators microorganisms into new cells, the synthesis of vital molecules, and electrons to obtain energy. This work was done with water from a pond and a mine pit of a disabled iron mining region, and techniques such as Biolog Ecoplate were used to compare the local microbial populations. This technique is based on measurements of the use of different carbon sources such as carbohydrates, polymers, amino acids, phenolic, carboxylic acid and amine / amides. These measurements enable the classification of microbial metabolic capacities, and consequently the functional diversity of communities. The result showed that both microbial communities, from the pond and the mine pit, have a similar Shannon index. However, since they come from different environments, they have different functional activities, with the pond sample showing greater evenness. In this work, it was also performed the isolation of bacteria from the mining environment, pond and mine pit. We obtained 6 isolated for the pond and 3 isolated for mine pit, showing similarity with the previous result of Ecoplate biolog.Tests with these isolated bacteria were performed in culture media and with different substrates to check the production of important biotechnological enzymes such as amylase, lipase, cellulase and phosphate solubilizing phosphate.The results were promising as the degradation efficiency of different compounds demonstrated that the populations are different, and that the microorganisms have a great ability to produce enzymes which can be of great biotechnological interest like cellulase, lipase and solubilizing phosphate.

P GCEH 21

Increasing salt concentration causes shift to the dominance of marine picocyanobacterial taxa in continental waters

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Members of the picocyanobacterial genera *Prochlorococcus* and *Synechococcus* contribute up to 80% of the planktonic primary production in open ocean ecosystems, and in many cases main phylogenetic groups (clades) denote ecologically distinct units. Members of the genus *Synechococcus* are also important in continental freshwaters and saline aquatic habitats, but freshwater phylotypes form phylogenetically completely distinct clades apart from those typically occur in the open ocean. Taxonomic identification of these small cyanobacteria is based on molecular biological techniques, because their distinctive morphological features are limited. Picocyanobacteria are also common in Central Europe: in freshwater, in soda ponds and in saline lakes. The studied shallow turbid soda pans are located in the Danube-Tisza Interfluve (Hungary) and are unique in Europe, while the studied sites in Transylvania (Romania) are deep salt lakes. Our aim was to determine the taxonomic composition of picocyanobacterial communities in freshwater (Lake Balaton, Hungary), in soda (Zab-szék, Hungary) and in saline lakes (Lake Tarzan, Lake Cabdic and Lake Ursu, Romania) on the basis of the comparative sequence analysis of the ribosomal ITS region and the 16S rRNA gene. It seems that salinity defines the ratio of marine and non-marine picocyanobacterial clades in these aquatic environments, since non-marine clades of the genus *Synechococcus* appeared in freshwater, soda lakes and in salt lakes having lower salt concentrations, but these taxa were absent in saline lakes having higher salt concentration. Interestingly, the *Synechococcus* community of all studied salt lakes was dominated by phylotypes characteristic to oceans and seas (marine clade VIII); the dominance of marine picocyanobacteria in continental waters were not reported previously.(This work was supported by the Hungarian Scientific Research Fund, OTKA PD 105407 and PD 112449.)

P GCEH 22

Microbial communities response to PAH contamination in coastal sediments

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Half of the human population in the world is concentrated on coasts. Consequently, coastal ecosystems ecologically support human activities and are highly impacted by anthropogenic pressure, and especially petroleum inputs. Hydrocarbons are likely accumulating in sediments, which harbor a great microbial diversity involved in major ecological services, among them : nutrient cycling and pollutants removal. Despite their ecological importance, we still struggle to understand the response of microbial benthic communities to hydrocarbon contaminations and their consequences on ecological services sustainability within coastal sediments.

Using high-throughput sequencing targeting 16S and 18S ARNr genes, we investigate the phylogenetic and functional diversity of benthic microbial communities in both impacted and pristine sediments samples from different geographical areas around Mediterranean sea, Gulf of Biscay and English Channel. Geographical origin of the samples was significantly driving both procaryotic and eukaryotic communities phylogenetic variability, as well as water column salinity. PAH contamination level and indicator of hydrocarbons origin (ie. Fluoranthene/Pyrene ratio) were not affecting both bacterial and eukaryotic phylogenetic diversity at the global level, while archaeal communities were significantly infuenced by both parameters. Archaeal taxons related to MBG-B and DHVEG-1 were indicators of high PAH contamination. Procaryotic predicted functional diversity response to PAH contamination was not following the same pattern as phylogenetic diversity.

Focusing on specific hydrocarbons degradation-related KEGG pathways revealed that several bacterial specific pathways were significantly more abundant in highly contaminated samples, while no difference was shown for archaeal pathways. Besides unravelling structuring processes of benthic microbial life in response to chronic hydrocarbon pollution, our results suggest that archaea may play an important role in the microbial response to PAH pollution.

P GCEH 23

Applying unconventional techniques for the laboratory cultivation of new bacterial strains

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For most of the bacteria, there are no pure laboratory cultures available, however many yet not cultivated microorganisms are potentially culturable even with the application of cheap and simple methods. A trial for enhancing the cultivation of bacteria was performed using samples taken from markedly different aquatic habitats (an acidic and humic peat bog lake, a highly polluted, deep, heliothermal, saline lake and an activated sludge reactor treating landfill leachate) applying in situ cultivation (polyurethane foam blocks impregnated with culture media), with the aid of special gelling agent (gellan gum), with special culture media (using pH, salt concentration and nutrient content values corresponding to parameters of the sampled water); and additionally with long-term incubation to support the growth of slow-growing bacteria. Based on the results of cultivation-independent analyses targeting the taxonomic composition of bacterial communities (next generation amplicon sequencing and terminal restriction fragment length polymorphism), it was shown that even incubation conditions supposed to be close to those present in nature provide strong selection pressure to bacteria. Nevertheless, pure cultures of several potential new genera and species were obtained, mainly belonging to the bacterial phyla Proteobacteria and Bacteriodetes, which supported the efficiency of the applied non-conventional cultivation methods. It seems that cultivation efficiency is not only significantly affected by medium composition, but also by the type of the applied gelling agent and incubation conditions.(This study was supported by the Romanian National Authority for Scientific Research CNCS-UEFISCDI, grant PN-II-RU-TE-2012-3-0319.)

P GCEH 24

Bacterial community succession in wood decomposition

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Degradation of plant remains is important in carbon and nutrient cycling. Next to fungi, the major wood debris decomposers, decaying wood is also colonized by bacteria. However, the relation between bacterial and fungal composition during wood decay is not yet well understood. Nevertheless, complex interactions between those two groups are expected. In previous wood blocks colonization studies strong fungal selection on bacteria was shown. It was shown that fungal communities undergo succession during the course of wood decay. However, so far, limited information is available on fungal effects on the bacterial community composition in wood decay under natural conditions.
In this study, we focused on bacterial community composition fluctuations in progressing pine wood decay. We hypothesized that the stage of decay will have an impact on the bacterial community structure. A strong selection of bacteria mediated by the dominant fungal species was expected. We further hypothesized that possible costly antagonism against bacteria is less prominent in late stages than during the stage with high amount of energy. Additionally, nitrogen is probably a limiting factor during early and middle stages of decomposition but not in the late phase.
We apply comprehensive assessment of decaying pine wood inhabiting microbial communities using pyrosequencing. Bacterial species richness and diversity increases from the early to late stage of decay, whereas fungal communities do not show this trend. Early stage of decay was overrepresented by *γ-proteobacteria*. *Rhodospirillales* and *Rhizobiales* (*α-proteobacteria*) were prominent in the middle stage, *Acidobacteria* Gp1 was high in in all stages.
Bacterial and fungal community co-occurrence analyses showed that complexity of co-occurrence of bacterial and fungal Orders was the highest in the middle stage of decay. Overall we observed absence of correlations for bacterial taxonomic groups with fungal community members.
We showed that the bacterial communities undergo less drastic changes comparing to the fungal communities. Bacteria respond to the stage of decay (wood density) but not to shifts in C/N content. We concluded that the shifts in the communities are a result of the changes in wood properties rather than related to fungal species itself.

P GCEH 25

The rise and fall of the rare biosphere: soil bacteria response to flash floods and desiccation in the desert.

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Soil water content greatly affects the microbial life, more so in desert environments where precipitation events are infrequent but intense. The effect of hydration on the soil microbial populations is being debated for many years and different scenarios were suggested. Yet, in-depth analysis of the changes in desert soil bacterial communities starting from the rain pulse through the desiccation of moisture from the soil were never reported, bared a few scattered snapshots of microbial profiles in arid soil during hydration or desiccation events.
We predicted that like macro-organisms hydration would supply micro-organisms with the much-needed water and increase their diversity and abundance, while desiccation would eliminate those that cannot survive water shortage and reduce both diversity and abundance of the bacterial community. To test our predictions we closely followed major rain events and the subsequent soil desiccation in the field and simulated hydration in arid soil columns linking bacterial diversity and interactions with soil water content.
Our data show that after a major rain events species richness and phylogenetic diversity plummet, while the total bacterial biomass is unscathed. Desiccation of the soil restores the bacterial diversity and richness while not affecting the abundance. Our controlled experiments showed that the changes in abundance and diversity wane when desiccation is rapid, suggesting that not only the amount of rain but also the air temperatures would impact the soil microbial community. Moreover, bacterial antagonistic interactions increased with hydration showing unidirectional competition against bacteria isolated from the desiccated soil.
We suggest that during hydration events, bacteria are involved in complex community dynamics; as the moisture in the soil increase gaps between the soil particles are bridged by water, enabling interactions and competition between formally separated communities leading to antagonistic interactions and a decrease in the overall bacterial diversity. However, when the soil desiccates the pores are again segregated and unique communities are established in each microhabitat thus stimulating bacterial dispersal.

P GCEH 26

Salinity and temperature effects on crude oil methanogenic biodegradation in river Tyne microbiota

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Methanogenic microbial degradation of crude oil is considered a dominant process occurring in anaerobic environments such as petroleum reservoirs. As a result, crude oil properties and composition is altered leading to a decrease in the oil quality with significant economic and ultimately environmental consequences. Environmental factors affecting crude oil biodegradation are still poorly understood. Temperature has been considered to play the main role in controlling microbial biodegradation in oil reservoirs. However, in such subsurface systems high salt concentrations may also key factor determining microbial growth and a plausible interaction between temperature and salinity has been proposed that could explain the occurrence of non-degraded petroleum reservoirs that have never been exposed to the threshold temperature of 80ºC thought to be responsible for reservoir pasteurization (Head et al., 2014). The present work aimed to study the combined effect of salinity and temperature on oil degradation rates in anaerobic microcosms using sediment from the River Tyne in Newcastle amended with crude oil under methanogenic conditions. In ongoing experiments methane production has been detected after 195 days at both mesophilic and thermophilic temperatures but not at the maximum temperature of 80ºC. For all temperatures the lag phases before detection of activity were longer and the production rates lower as a function of increasing salinity. Nevertheless, methanogenic activity has been detectable relative to controls at 120 gL^{-1} NaCl (so far only for microcosms incubated at 40ºC). To date unequivocal evidence of methanogenic oil degradation has been observed at 20ºC and 40ºC at the lowest salinity tested (15 gL^{-1} NaCl) but experiments are ongoing and our preliminary data indicate that longer time periods may be required to see activities at higher temperatures and salinities. These results offer insights to the microbial limits of microbial communities and environmental constraints governing crude oil biodegradation in anaerobic environments with important implications for petroleum reservoirs environments.
-Head IM, Gray ND and Larter SR (2014) Front. Microbiol. 5:566.

P GCEH 27

Drinking water microbiome: from groundwater to the tap

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Drinking water quality is a public health concern worldwide. Growing evidences depict drinking water as a complex matrix, in which a wide diversity of microorganisms interact in a dynamic network. Recent studies reveal that drinking water treatment process can affect the microbiome structure [1,2,3]. In particular granular activated carbon filters seem to play a crucial role in shaping bacterial community downstream the treatment processes [2]. Moreover the occurrence of antibiotic resistance genes in water is becoming an issue of great interest as the mobile resistome can easily spread among species [1,4]. Molecular techniques can give a deeper knowledge, going beyond the limit of culture-dependent methods [5].
In this study we standardized a new pipeline for microorganisms concentration, DNA extraction and amplification, suitable for molecular analysis and optimized for High-Throughput Sequencing (HTS) approaches, in order to analyze drinking water microbiome.
We collected samples in a water treatment plant in Milan (Italy), at different steps of the potabilization processes: raw water from the aquifer, treated water after granular activated filters and water after the final step of chlorination. We analyzed the presence and the relative abundance of bacteria and eukaryotic microorganisms across the water treatment plant. Furthermore the presence of specific antibiotic resistance genes was detected and quantified with Real Time PCR, at each step of water treatment process. Since molecular techniques are unable to differentiate between viable and nonviable microorganisms, live/dead ratio was estimated using SYTO9/propidium iodide staining coupled with microscopy visualization. These analyses are integrated in a broader study characterizing microbiome structure variability using HTS techniques, in order to better understand this complex ecosystem. The results agree with those obtained in the few recent studies published till now and can help to unravel the dynamics underlying water microbiome changes.

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P GCEH 28

Impact of local and global metal contaminations on microbial communities of Pyrenean lakes

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Mountain lakes are both a natural heritage of the Pyrenean massif and natural receptors allowing the detection of global changes or anthropogenic contaminations. Recent studies showed that the integrity of this heritage is threatened either by metal deposition from the atmosphere, either by metal contamination from past mining activities. Unfortunately, to date no study has been undertaken to determine the consequences of such contaminations on the biodiversity and functioning of these ecosystems. Because of their critical ecological role in lake ecosystems (i.e. biogeochemical recycling of major nutrients), metal's impacts on microbial communities (ie Bacteria, Archaea and Eukaryotes) may potentially have system-wide implications. Hence, the primary goal of the work described here was to compare microbial community structures in water column and sediment samples collected from mountain lakes with differing levels of metal contamination. Our results showed that some heavy metals and metalloids concentrations such as As, Cd, Cu and Pb were way above ecological risk indices in most locally and atmospherically impacted lakes. In agreement, we observed an erosion of the biodiversity in

atmospherically contaminated lakes. We also found that metals, and particularly Pb, Zn, Sb and Tl, had a greater effect on microbial community composition than physico-chemical or geo-morphometric parameters. This was particularly true for eukaryotic assemblages, which seemed less resilient than prokaryotic assemblages. Overall, we identified for each domain of life potential biomarkers of metal contaminations. These results suggest that anthropogenic metal contaminations reaching mountains lakes have modified the biodiversity of these remote habitats and potentially altered the ecological services they sustain.

P GCEH 29

Soft coal mining and mineral leaching - bacterial life in slag deposits

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Introduction: The leaching of minerals such as vitriol and alum from soft coal for industrial purposes was common practice until the end of the 19th century. Significant amounts of leached-out-slag as by-product were dumped close to former soft coal mines where they are still shaping the landscape.
Objective: Research about the microbial life in slag deposits, an anthropogenic extreme habitat.
Materials and Methods: Slag samples originating from alum leaching were collected in Holtorf (Germany) (50°74'N, 7°17'E). Bacterial diversity was studied by iTag sequencing and quantitative real-time PCR of 16S rRNA genes. Elemental analyses were carried out by combustion analysis (C, H, N, S) and ICP-MS (Al, Ca, Fe, Mn, P).
Results: Alum-forming elements (Al, Fe, S) were significantly enriched in slag samples. The concentrations of Fe and S, for instance, were increased up to 60-fold (44-48 mg/g dry weight) compared to the reference soil (0.8-4 mg/g dry weight). Slag samples were acidic (pH ~3.5). Bacterial diversity and community evenness in these samples were low as indicated by the Chao1 and Shannon indices and 16S rRNA gene copy numbers were ten times lower than in the reference soil. Beta diversity analyses based on Jensen-Shannon-Divergence revealed a clustering of slag bacterial communities distinct from those of the reference soil. Major phylum-level groups were *Acidobacteria* (12-27%), *Actinobacteria* (20-35%), *Chloroflexi* (20-27%), and *Alphaproteobacteria* (13-18%). At the lower taxonomic level, the analysis revealed that uncharacterized groups dominate the bacterial community structure: DA052 (up to 16%), KF-JG30-18 (up to 3%) [both *Acidobacteria*], TM214 (5-20%, *Actinobacteria*), DA111 (7-8%, *Alphaproteobacteria*), and JG37-AG-4 (15-20%, *Chloroflexi*). These groups were previously detected in peatlands and anthropogenic pollution sites, but only in low abundances.
Conclusions: Slag deposits originating from mineral leaching represent an unusual and rather extreme environment favoring the presence of various so-far-uncharacterized microbial groups. Current research shall shed more light on the ecophysiology of these populations using metagenomics and targeted cultivation strategies.

P GCEH 30

The effect of fish farming on sediment microbial community

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Fish farming can affect the microbial community in the sediments below the farms by several ways. Aquaculture can cause eutrophication and oxygen depletion in the sediments by introducing nutrients from uneaten fish feed and fish feces. Occasionally fish are treated with antibiotics and the antibiotics also end up in the sediments. We collected sediment samples from a fish farm and a control site at three time points spanning over seven years for metagenomic sequencing. The taxonomic and functional gene compositions were studied to see the detailed effects of aquaculture on the microbial community and functions. Antibiotic resistance genes were annotated to detect possible enrichments due to the use of antibiotics. The farm and control sediments were different both on taxonomic and functional gene compositions and clustered based on the sampling site showing that farming changes the bacterial community in the sediments. We found anaerobic sulfate-reducing bacteria and methanogenic archaea enriched in the fish farm sediment, suggesting anoxic conditions probably caused by eutrophication. Antibiotic resistance genes were more abundant in the farm sediments, which is in line with our previous results with qPCR methods, and this might pose a threat of antibiotic resistance spread to pathogens

P GCEH 31

A framework to understand the ecological mechanisms mediating microbial community assembly along successions

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Ecological succession and the balance between stochastic and deterministic processes are two major themes within microbial ecology, but these conceptual domains have mostly developed independent of each other. Here we provide a framework that integrates shifts in community assembly processes with microbial primary succession to better understand mechanisms governing the stochastic/deterministic balance. Synthesizing previous work, we devised a conceptual model that links ecosystem development to alternative hypotheses related to shifts in ecological assembly processes. Conceptual model hypotheses were tested by coupling spatiotemporal data on soil bacterial communities with environmental conditions in a salt marsh chronosequence spanning 105 years of succession. Analyses within successional stages showed community composition to be initially governed by stochasticity, but as succession proceeded there was a progressive increase in deterministic selection correlated with increasing sodium concentration. To evaluate scale-dependency, we examined turnover in community composition among successional stages, which provided a larger spatiotemporal scale relative to within-stage analyses. Change in soil organic matter concentration was the strongest predictor of both the type and relative influence of determinism, suggesting scale-dependency in the mechanisms underlying selection. To better understand mechanisms governing these patterns, we developed an ecological simulation model that revealed how changes in selective environments cause shifts in the stochastic/deterministic balance. Finally, we propose an extended—and experimentally testable—conceptual model integrating ecological assembly processes with primary and secondary succession. This framework provides *a priori* hypotheses for future experiments, thereby facilitating a systematic approach to understand assembly and succession in microbial communities across ecosystems.

P GCEH 32

Bacterial diversity and chemical pollutants investigation in ice core of Campbell Glacier in Northern Victoria Land in Antarctica

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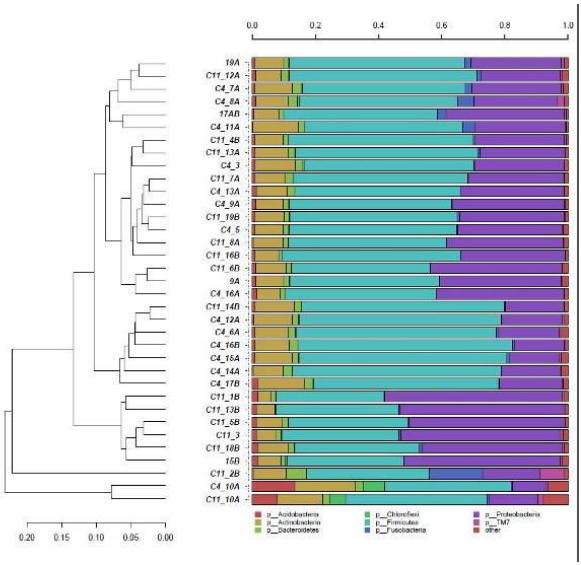
Introduction: This study was performed on ice cores drilled in two area of the Campbell Glacier which ends with floating ice tongue about 130 km of length, located in Northern Victoria Land in Antarctica.
Objectives: 1. Detection and identification of living bacteria still present in ice core. 2. Microbiome identification by amplification of 16S rRNA regions coupled to the High-throughput sequencing technologies (HTS). 3. Preliminary investigation of the presence of two classes of persistent organic pollutants: polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs).
Materials and methods: Ice core drilled were drilled in two area Camp4 74° 13' 34"S, 163° 58' 58"E, 8,94 m of depth and Camp11 73° 43' 45"S, 163° 18' 31"E, 12,30 m of depth. The ice cores (36samples) were handled under aseptic conditions. The water obtained from melted ice was used for analysis of: strains isolation, DNA extraction for HTS from the filter membrane and investigation of PAHs and PCBs performed with a gas chromatograph coupled to a mass spectrometer (GC-MS).
Results: Living bacteria were recovered belonging to the following genera: *Stenotrophomonas*, *Chryseobacterium*, *Zoogloea*, *Duganella* , *Janthinobacterium*, *Agrococcu*s, *Micrococcus*, *Lysinibacillus*, *Paenibacillus*, *Bacillus, Microbacterium, Sphingomonas, Pedobacter* , *Kocuria*, *Arthrobacter*, *Rothia*. Preliminary data obtained by HTS sequencing allowed an estimate coverage more than 97% of the total microbial communities. The distribution at phylum level revealed the dominance of Firmicutes, followed by Proteobateria and lesser extent was the presence of Actinobacteria. The family distribution in 15 ice core samples showed a relevant amount of Oxalobacteaceae, Lachnospiraceae and in other 13 ice core were detected Clostridiaceae, Pasteurellaceae, Moraxellaceae. The analysis of PCBs and PAHs, revealed the presence of the PCB congener 52 in three of the four preliminary samples (13-19 ng L^{-1}) and of the PCB congener 118 in two of the four

preliminary samples, values near the LOD. PAHs in the ice samples: the most representative was phenanthrene, ranging 0.20 - 0.83 µg L^{-1}, followed by pyrene present as well in all the samples but at values near the LOD.
Conclusion: The results provide information of microbiome and pollutants in a glacier of Antarctic continent.
Figure 1



P GCEH 33
Evidence for shift from Acidobacteria to Proteobacteria dominance in soil profile of boreal acid sulphate soils in FInland
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Introduction: Acid sulphate (AS) soils located along the Baltic Sea coast may contain large carbon and nitrogen stocks in subsoil horizons. If these soils are drained and used for agriculture, large fluxes of carbon and nitrogen gases could therefore occur.
Aims: We aimed to compare bacterial community composition of drained AS soils in 4 soil horizons in comparison to drained non-AS soil assessed by 16S-pyrosequencing. We sampled three AS soils - Patoniitty (P), Söderfjärden (S), and Ylistaro (Y) with the different cultivation history, and one non-AS, Alaniitty (A). A and P soils are located in close vicinity, on the research farm of the University of Helsinki.
Materials & Methods: Four different soil horizons were studied: Ap, Bg, BC, and C horizons. AS and non-AS soils differed in the amount of organic C, total N, sulphur species, as well as in pH and microbial activities in the C horizons. In topsoils, RNA-based active bacterial community was studied too.
Results: Bacterial communities in Ap horizons were dominated by Acidobacteria, followed by Proteobacteria (α- and β-), Planctomycetes, Gemmatimonadetes, and Chloroflexi. In deeper horizons (B and BC), the relative abundance of Proteobacteria increased at the expense of Acidobacteria. The differences in bacterial communities between AS and non-AS soils increased, being characterized by the enrichment of Gemmatimonadetes and Chloroflexi in non-AS soil; and Actinobacteria, Spirochaetes and Cyanobacteria in AS soils. The highest shifts has been observed in C horizons, where the higher abundance of Candidate division OP9, Planctomycetes, and Chloroflexi has been observed in AS soils in comparison to non-AS. In topsoil, RNA-based bacterial community was dominated by genera: *Pirerulla*, *Gemmata*, *Planctomyces*, *Caldilinea*, *Phaselicystis*, *Candidatus Solibacter*, *Caenimonas*, and *Haliangium*.

Conclusions: As boreal AS soils have developed from sulphide-bearing sediments of Baltic Sea, it may indicate the remains of the bacterial communities, in particular Candidate division OP9 putatively associated with anaerobic methane oxidation coupled with sulphate reduction.

P GCEH 34

Large Scale Spatial Analysis of Bacterial Communities in Lake Sediments, the Role of Physico-Chemical Parameters, Spatial Distance, Land Cover and Tropical Storms

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Sediment and soils are among the most microbial diverse ecosystems on the Earth. While a variety of soils and sediments have had some of their DNA sequenced, much remains to be explored in terms of how these communities are structured, the extent of their interactions with their physical and chemical environment, and their role in ecosystem functioning. The spatial distribution of bacterial communities inhabiting the sediments is highly heterogeneous at different spatial scales, but is still mostly unexplored. Some studies have suggested links between the spatial diversity of soil microbes and soil physicochemical parameters (*e.g*., relationship between soil pH and *Acidobacter* abundance). In this project, we hypothesize that heterogeneity of the bacterial community composition varies at the same scale level of the heterogeneity of sediment chemical properties. Here, we focused on the large scale (km) diversity. The large scale physical and chemical characteristics that we hypothesize influence microbial communities in lake sediment at the kilometer scale are land cover, climate, pH, and salinity. We tested this by examining the spatial distribution of bacteria and physical and chemical parameters in sediment of the second largest brackish lake in the world (Chilika Lake, India). Seventy-two samples (24 stations, 3 seasons-winter, rainy and summer) of sediments from Chilika Lake were analyzed by 16S rRNA gene pyrosequencing. Land cover analyses were performed using satellite images and a digital elevation model with geographic information system (GIS), and a large set of physico-chemical analyses (e.g., pH, turbidity, salinity, conductivity) were also performed on the water column over the sediment. After a hurricane passed near the lagoon in 2011, more samples were collected to see the impact of the tropical storm on the spatial distribution of bacteria in the sediment. The results of 16S rRNA gene analysis and physical and chemical parameters used with the spatial analysis demonstrated clear spatial relationships between physico-chemical parameters (salinity), land surfaces (drainage area, type of vegetation…) and extreme events, and the distribution of sediment microbial communities.

P GCEH 36

Spatial pattern of bacterial diversity in a site with mixed and uneven contamination, and assessment of rhizoremediation potential

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The SIN Caffaro is a large polluted site of national priority located in the Northern Italy, originated by the activities of the former Caffaro s.p.a. chemical factory. The soil in the site presents a mixed contamination of halogenated Persistent Organic Pollutants, particularly polychlorinated biphenyls, and heavy metals in variable concentrations, uneven distributed in the area and often exceeding the safety values. The use of plants to extract and modify the pollutants (phytoremediation) together with root associated microbes to i) degrade or modify the pollutants (rhizoremediation) or ii) support plant growth (plant growth promotion, PGP) has recently arose as a promising approach for bioremediation. The high concentration of pollutants could therefore be considered as a gradient of environmental selection toward the resident community potentially able to support soil remediation.

In this context, we collected 63 soil samples from three different areas in the site to a depth of 1 meter, which were chemically and microbiologically characterized. A DNA-based fingerprinting approach was applied to describe the bacterial community's structure, which proved to be significantly different according to the area and depth of collection. Through a statistical approach, we tested the influence of selected environmental parameters, showing that the concentration of different classes of pollutants was significantly related to the microbiome structure.
Furthermore, the rhizosphere of three autochthonous plant species was collected in the most contaminated area of the site. The overall bacterial community was studied by 16S rRNA pyrosequencing and a collection of bacterial strains was in parallel established and tested *in vitro* and *in vivo* for PGP potential. The results showed that the rhizosphere-dwelling microbiome was highly similar between the plant species, in terms of both phylogenetic diversity and PGP potential, confirming the existence of a strong selective pressure given by the pollution profile rather than the plant species.
Overall, this work highlighted the occurrence of distribution patterns in bacterial populations related to gradients in soil pollution, and showed the intrinsic potential of the highly contaminated soils at the Caffaro site for rhizoremediation potential.

P GCEH 37
Diversity and small-scale distribution of microbial communities in Patterned Grounds
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Patterned Grounds (PGs) are geometric features formed by cryoturbation on soils affected by periglacial processes. Although many studies described PGs morphology and formative processes, microbiological aspects that may play important roles in nutrient availability, dynamics and stabilization in ecosystem evolution are yet greatly unexplored.
In this study, we focused on sorted or unsorted circles showing a concentric textural sorting, from four sites, characterized by different lithotypes, in the North-western Italian Alps. In order to give insights on microbiological processes affecting PG features and to find microbial markers potentially useful to explain and predict the evolution of cryoturbated ecosystems, abundance, structure and distribution of bacterial, archaeal and fungal genetic markers and functional genes were measured and correlated to chemical soil parameters.
Microbial structure analysis revealed a relatively homogeneous community within circles, but also among different PGs, with the presence of several phylotypes previously observed in other PGs and cold environments. Only for Archaea stronger differences in community composition on small-scale were detectable. On a quantitative point of view, microbial populations showed a clear concentric distribution, well correlated to soil C:N ratio, influenced by lithology of parent material and coherent with trends of physicochemical parameters within PG circles.
These first results highlight the importance of cryoturbation in shaping microbial communities in permafrost soils, particularly affecting their quantitative distribution. If, on one hand, this could be linked to a mere mechanical sorting activity exercised on microbial biomass by soil and water mixing, on the other the presence of a concentrically differentiated archaeal population and the absence of a clear concentric trend in functional genes suggest a more complex situation, with microorganisms actively involved in ecosystem modelling and evolution.

P GCEH 38
Changes in bacterioplankton community and carbon cycling rates along the Subtropical Frontal Zone off New Zealand suggest preferences in metabolic lifestyles associated to different water masses
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Marine heterotrophic bacterioplankton play a central role in ocean carbon cycling. As such, identifying the factors controlling these microbial populations is crucial to fully understanding carbon fluxes. The Southland Front is the local expression of the Subtropical Frontal Zone, and is associated with the "Munida transect", which has an extensive record of physicochemical data amassed since 1998. Two major water masses, representative of around ¾ of the Earth's surface waters, compress in this area creating very strong spatial gradients in physicochemical parameters. These two colliding circumpolar water masses possess contrasting nutrient regimes (macronutrient-limited [N, P, Si]) subtropical waters and the micronutrient-limited high-nutrient low-chlorophyll (HNLC; i.e. Fe) sub-Antarctic waters. Our objective was to determine bacterioplankton community shifts associated to this gradient, and corresponding changes in metabolic lifestyles associated with adaptation to

contrasting nutrient regimes. Bacterioplankton activity (heterotrophic production, respiration), community structure (via 16S rRNA gene analysis) and functional composition (by metagenomic analysis using Illumina sequencing) were determined along a transect crossing three water masses (i.e., Subtropical waters [STW], Sub-Antarctic waters [SAW] and neritic waters [NW]) with contrasting nutrient regimes across the Subtropical frontal zone. A strong gradient in bacterioplankton carbon cycling rates was observed along the Subtropical frontal zone, mainly linked to the HNLC conditions of SAW. Shifts in cycling rates coincided with distinct microbial populations hosted in each water mass. Both rates and community structure correlated to specific metabolic potentials. Further, comparison of surface with deep (500 m) waters demonstrated structuring of microbial communities across multiple scales. Shifts in microbial populations suggest that organisms associated with each water mass have evolved different metabolic strategies for coping with their energy and carbon requirements.

P GCEH 39

Cyanobacteria, too small to sink? Relative and absolute contribution of cyanobacteria to the carbon export in the Sargasso Sea

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The ocean sequesters more than ¼ of the carbon released by anthropogenic action every year, and oligotrophic oceans, such as the Sargasso Sea, are responsible for about the 60% of the global carbon export. Pico and nanophytoplankton, such as cyanobacteria, dominate the primary production in the Sargasso Sea, with *Synechococcus* being more abundant in the winter, while *Prochlorococcus* in the summer. However, very little is known about their contribution to the carbon export. We will report data on the relative and absolute contribution of these cyanobacteria to the flux of particulate matter as collected with shallow particle traps (150 m). We applied two different molecular techniques: 454 pyrosequencing targeting the 16S rDNA (V4 region) and quantitative Polymerase Chain Reaction targeting the 23-16 rDNA internally transcribed spacer region. We collected seawater samples within the euphotic zone and compared them to the sinking particles at 150 m during the spring and the summer of 2012 at the Bermuda Atlantic Time-series Study station and in the surrounding mesoscale eddies. We found that *Synechococcus* was overrepresented in trap libraries compared to the water column. On the contrary, *Proclorococcus* was found to be under-represented in the sinking particulate matter. Also, we find, that different strains of *Synechococcus* dominate in the spring vs summer, and we will show how these different strains contribute quantitatively to the downward particle flux in the Sargasso Sea in both seasons. In summary, it is predicted that the biological carbon pump is changing due to anthropogenic activity, and that *Synechococcus* will become more abundant in a future warmer ocean. The Sargasso Sea is the perfect in situ laboratory to study the contribution of pico primary producers on the carbon export today, and our study will establish a baseline that will enable us to better predict the consequences of a changing community on the biological carbon pump in a future ocean.

P GCEH 40

Influence of environmental pH on thaumarchaeal diversification

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Ammonia-oxidizing Thaumarchaeota are abundant in many ecosystems, including the soil, and perform a key function in the global nitrogen cycle. Previous high throughput sequencing analysis of the ammonia monooxygenase gene *amoA* has demonstrated that pH is the major driver of thaumarchaeal niche specialization and community structure of these organisms. While many studies have examined the adaptive distribution and ecophysiology of extant Thaumarchaeota, the evolutionary rise of these prokaryotes to a position of ecological dominance in many habitats has never been considered. Therefore, we characterized thaumarchaeal diversification with respect to ancestral reconstructions of soil pH adaptation, employing state-of-the-art comparative phylogenetic methods based on extensive *amoA* and 16S rRNA sequence data. Our analysis shows a striking increase in lineage diversification rates during early thaumarchaeal evolution that was coupled to major pH adaptation events. While radiation of eukaryotes usually involves an explosion in diversity followed by a decrease in diversification rate, the high initial rate of diversification of Thaumarchaeota remained globally stable during the last 400-700 Ma, resulting in a high level of thaumarchaeal diversity nowadays. Among the several environmental factors tested,

adaptation to pH appeared to correlate nicely with the thaumarchaeal diversification pattern, indicating the primordial influence of such environmental gradient on prokaryotic adaptation and evolution.
Overall, this study highlights the surprising pattern of thaumarchaeal diversification together with the important role played by pH specialization in thaumarchaeal evolution and is the first of its kind to link diversification with phenotypic adaptation in a prokaryotic phylum. This study provides a framework for comparing dynamics of evolutionary processes across the tree of life and to better understand the diversification processes and past molecular innovations in archaea.

P GCEH 41

Effects of Saharan Dust Storms on the Diversity and Composition of the Microbiota in Mountain Glacier Snow

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Introduction. Dust particles in desert storms can move over great distances in the atmosphere and the uplifted microorganisms may survive to long-range transport. African desert storms cross the eastern Mediterranean and inject a large pulse of microorganisms into the European air, expanding their biogeographical range. The impact in anthropized areas may be difficult to study because of the complexity of the urban scenario. On the contrary, remote ecosystems, such as mountain glacier, are interesting case studies because more sensitive to exogenous inputs.
Objectives. The aim of this study was to characterize the microbiota in atmospheric particulate matter during Saharan dust storms and in snow with and without desert dust deposition.
Materials & methods. Coarse particulate matter (PM10) was harvested during an intense desert storm in Central Italy, while snow samples came from the Calderone glacier (Gran Sasso d'Italia, 2700m asl), the southernmost in Europe and the only one in the Apennines. Diversity and composition of the microbial communities were investigated using Illumina next generation sequencing (NGS) of amplified 16S rRNA gene fragments.
Results. Models of air mass backward trajectories together with decreasing values of PM2.5/PM10 ratio indicated the occurrence of a dust storm event originated from the Sahara desert. Snow samples with desert dust deposition were characterized by high enrichment factor values for Mn, Pb and Zn. Illumina NGS analysis showed that PM10 and snow samples featured complex microbial communities with peculiar bacterial phylotypes. As expected, snow samples were largely characterized by the presence of bacterial populations known to be present in cold environments. Interestingly, many of the sequences retrieved in the PM10 collected during the desert storm and in the dust-impacted snow matched with bacteria typical of soil and/or desert environments, namely those belonging to the genera *Acidovorax, Stenotrophomonas, Sphingomonas, Hymenobacter, Rugamonas*, and *Massilia*.
Conclusion. The occurrence of bacterial populations typical of desert environments confirmed the impact of desert dust deposition on Calderone Glacier snow and indicated the potential capacity of airborne microbiota originated from the Saharan desert to colonize remote mountain environments.

Oral presentations

O SOIL 1

Plant root exudates shape the diversity of denitrifying bacteria and stimulate their activity

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Denitrification process occurring in plant rhizosphere allows the return of fixed nitrogen to the biosphere. It is well known that the major factors regulating denitrification can be modified in the rhizosphere: nitrate concentration (via the N assimilation by plants) and oxygen partial pressure (via root respiration) are decreased, whereas C availability (via rhizodeposition) is generally increased (Mounier *et al.,* 2004). The impact of the rhizosphere on denitrifying activity has been previously reported (Henry *et al.,* 2008). However, little is know about how denitrification is regulated in the rhizosphere. Hence, understanding the regulation of denitrification and the role of organic carbon, released through plant root exudation, in the production of N_2O and in shaping denitrifying bacterial diversity is of global importance and offers the opportunity for managing soils to lower net N_2O emission.

In the present study, we question whether root exudates from four plant species; *Triticum aestivum*, *Brassica napus*, *Medicago truncatula*, and *Arabidopsis thaliana* cultivated in the same soil, may have an impact on the structure and function of denitrifying bacteria by targeting the expression of nitrite reductase genes (*nirK* & *nirS*) by high throughput sequencing technics and by measuring denitrification activity in the root tissue and in the root-adhering soil (RS). In addition, to understand how each plant species modulate denitrification activity mainly in the root compartment, we determined the rate of nitrogen uptake from nitrate and from ammonia for each plant species.

We provide evidence that each plant species shapes denitrifying bacterial community structure. The N_2O production rates measured by adding nitrate as sole nitrogen source, revealed a higher denitrifying activity on plant root compartments where root exudation is more important than in the RS for all studied plants. Denitrifying activity was higher on *T. astivum* and *A. thaliana* root systems that showed a lower preferential uptake of NO_3^- and lower on *M. truncatula* and *B. napus* which present no preferential uptake and a greater uptake capacity for NO_3^- respectively. This study demonstrated that denitrification process was significantly activated and thus positively regulated in the root compartment.

O SOIL 2

The role of *Desulfitobacterium* spp. in the global network of *O*-demethylation in soil

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Introduction: *Desulfitobacterium* spp. are strictly anaerobic bacteria that are typically described as reductively dehalogenating bacteria [1]. Their presence in the environment has mostly been reported at sites contaminated with halogenated compounds. An additional metabolic trait of these bacteria is the *O*-demethylation of phenyl methyl ethers [2], which are products of aerobic lignin degradation and abundant carbon and energy sources in forest soils. This points to a potential, yet unexplored ecological niche of desulfitobacteria and their presence in uncontaminated environments.

Objectives: To elucidate the role of *Desulfitobacterium* spp. among other soil bacteria in the *O*-demethylation of phenyl methyl ethers within the network of fungal and bacterial lignin decomposition.

Methods: Growth experiments were used to test the ability of *Desulfitobacterium* spp. to demethylate selected phenyl methyl ethers in the presence of electron acceptors that naturally occur in soils. Soils were sampled in the vicinity of Jena and methylotrophic bacteria were enriched by using the *O*-demethylation of syringate, coupled to the reduction of thiosulfate, as the growth-selective process. *Desulfitobacterium* spp. were detected via qPCR and the soil and enriched microbial communities were characterized via Illumina MiSeq technology.

Results: *O*-demethylation was found to be a common trait of the genus *Desulfitobacterium*. The *O*-demethylation of 4-hydroxyanisole, syringate, vanillate and isovanillate could be coupled to the reduction of fumarate, nitrate, thiosulfate and Fe(III). It was possible to enrich *Desulfitobacterium* spp. from forest and grassland topsoils by exploiting their methylotrophic metabolism. The quantification of 16S rRNA gene copies revealed their initial enrichment during the early cultivation stages, followed a gradual loss. Community analyses via Illumina MiSeq technology revealed a co-enrichment of acetogens as the main cause for this phenomenom.

Conclusion: The results point to a methylotrophic lifestyle of *Desulfitobacterium* spp. in uncontaminated forest and grassland topsoils and to their involvement in the *O*-demethylation of phenyl methyl ethers among acetogens.

[1] Villemur *et al*. (2006) *FEMS Microbiol Rev* 30: 706-733.
[2] Neumann *et al*. (2004) *Arch Microbiol* 181: 245-249.

O SOIL 3

The power of the rare: sulfate reduction in an acidic peatland is driven by small networks of natively low abundant bacteria

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Peatlands are regarded primarily as methanogenic environments significantly contributing to global methane emissions. Little attention is given to the fact that dissimilatory sulfate reduction is maintained by a hidden sulfur cycle in these low-sulfate environments, with sulfate reduction rates being comparable to marine surface sediments. To deepen our understanding of sulfate reducers in peatlands, anoxic peat slurries were supplemented with typical degradation intermediates of organic matter at *in situ* concentrations and either stimulated with low amounts of externally supplied sulfate or incubated under endogenous conditions. Changes in the microbial community were monitored by 16S rRNA gene and cDNA amplicon sequencing and correlated to substrate and sulfate turnover. OTUs most abundant in the native community (*Acidobacteria, Actinobacteria, Alphaproteobacteria, Planctomycetes*) showed no significant response to sulfate amendment. In contrast, small networks of natively low abundant bacteria strongly correlated with bulk sulfate turnover under lactate, propionate, and butyrate. Among the responsive OTUs affiliated to recognized sulfate reducers, members of the genera *Desulfomonile* and *Desulfovibrio* (*Deltaproteobacteria*) responded specifically to one of these three substrates, while a *Desulfopila* OTU (*Deltaproteobacteria*) and a *Desulfosporosinus* OTU (*Firmicutes*) were always responsive exhibiting a generalist lifestyle. Interestingly, the *Desulfosporosinus* OTU markedly increased its 16S rRNA and thus ribosome content but stayed at low abundance throughout the incubation period. This likely mirrors its ecological strategy also in the natural peat soil. Parallel sequencing of a metagenome enriched by DNA-stable isotope probing allowed almost complete reconstruction of the *Desulfosporosinus* population pan-genome and confirmed functional properties of this peatland sulfate reducer. The small networks of responsive OTUs always contained at least one member not affiliated to recognized sulfate reducers (e.g., *Alphaproteobacteria*) indicating possible metabolic interaction partners. In conclusion, our results show that the activity of low abundance microorganisms can have a profound effect on biogeochemical cycling and control of greenhouse gas production.

O SOIL 4

***Miscanthus x giganteus* crops improve microbial patrimony in polluted soils**

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The discharge of urban waste water on farm land has led to a widespread introduction of pollutants into our environment, locally causing acute and diffuse contamination of soils. As a consequence, contaminated soils are unsuitable for crop production and need rehabilitation. One strategy is the establishment of energy crops such as *Miscanthus x giganteus*, a C4 grass used for biofuel production. Although the economical potential of this crop is known, its effects on soil biological properties remains unclear.

The aim of this study was to assess the effects of miscanthus perennial cropping on the microbial properties in a contaminated soil. The work is based on an experimental field site close to Paris irrigated for more than hundred years by raw wastewater (Pierrelaye, France). Soil microbial communities were characterized in terms of abundance, diversity and composition with metagenomic tools (soil DNA extraction, estimation of microbial molecular biomass and high throughput

sequencing of 16S and 18S ribosomal genes). The impact of perennial crops on microbial communities was assessed by (a) a synchronic study comparing communities between a field under miscanthus for four years and a field managed under conventional cereal cropping (tillage, crop rotations); and by (b) a diachronic study monitoring communities during a two years period of growth of miscanthus following implantation.
Our results showed that the composition of the microbial community at the site was indicative of polluted state of the soil, with populations involved in polyaromatic hydrocarbons and hormone degradations, and metallic resistance. Perennial cropping significantly increased fungal diversity, richness and equitability. Such perennial cropping stimulated bacterial and fungal *genera* known to live in association with roots and/or to degrade easily organic matter via copiotrophic attributes. Altogether, our results show that the establishment of miscanthus may represent a good strategy to stimulate microbial resources in polluted sites, hence favoring their rehabilitation.

O NON 1

Dissimilatory nitrogen reduction of soil fungal community members and their possible contribution to N_2O production in soil

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Denitrification, the reduction of nitrate or nitrite over nitric oxide (NO) and nitrous oxide (N_2O) to dinitrogen gas (N_2) has been well studied in bacterial communities over the last decades, while archeal and fungal community involvement has been less well explored. Several recent microcosm studies suggest the important role of fungi in N_2O emissions from various soil environments, thus contributing to the accumulation of this greenhouse gas in the atmosphere (Chen et al. 2014) (Ravishankara et al. 2009). Although a denitrifying, N_2O producing fungus was described at the beginning of the 1990's (Shoun 1991), there is still little knowledge on dissimilatory nitrogen reduction in the fungal kingdom and there is a lack of functional gene sequences that would help interpreting their role in the environment. By performing a screening of 600 isolates of soil-inhabiting fungi on nitrate and nitrite usage as a terminal electron acceptor and monitoring the production of NO, N_2O and N_2 gases in controlled-atmosphere pure-culture conditions, we have determined the taxonomic distribution of N-reducing fungi and rate constants of NO and N_2O production. The influence of low pH, a key parameter leading to higher N_2O emissions by bacterial denitrifiers, has been examined. In contrast to former studies on fungi, this study monitored NO production and rates in addition to those for N_2O. Genomic sequences from isolates involved in NO and N_2O production will be used in a metagenomic study to study nitrogen transforming fungal communities.

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Microbial diversity and functioning in the soil ecosystem

O NON 2

Growing up in a tough neighbourhood: adaptations of ammonia oxidising archaea enabling growth at low pH

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Ammonia oxidation is the first and rate-limiting step in nitrification and is performed by two distinct groups of microorganisms: ammonia-oxidising archaea (AOA) and bacteria (AOB). Approximately 50% of the world's arable soil is acidic and previous research identified mechanisms, such as ureolysis and aggregate formation, that enabled the growth of neutrophilic AOB under acidic conditions. However, it is now thought that AOA dominate ammonia oxidation activity in low pH soils, with the cultivation of the acidophilic ammonia oxidiser *Nitrosotalea devanaterra* providing the most parsimonious explanation for observed high rates of nitrification at low pH. *N. devanaterra* is a representative of a lineage that dominates AOA communities in many acidic soils globally and is the only known ammonia oxidiser that does not grow at neutral pH. However, a major paradox is that ammonia, rather than ammonium, is thought to be its substrate for energy generation, despite ammonia being present at extremely low concentrations at low pH.

To identify mechanisms that facilitate this unique phenotype, the sequenced genome, transcriptional activity and lipid content of *N. devanaterra* were analysed. Previously proposed mechanisms for AOB acidophilic growth in soil are not essential for archaeal ammonia oxidation at low pH. Perhaps surprisingly, analyses also indicate that the ammonia oxidation machinery is, at a fundamental level, the same as that proposed for neutrophilic AOA. However, unlike AOB, AOA may possess an efficient substrate acquisition system for ammonium (rather than an ammonia) and an array of putative pH homeostasis mechanisms enable *N. devanaterra* to oxidise ammonia at low pH. Transcriptional activity of candidate genes encoding for these novel mechanisms was demonstrated by RT-qPCR, and pH and homeostasis genes of *N. devanaterra* were differentially expressed in response to external pH. As organisms belonging to the genus *Nitrosotalea* are the dominant ammonia oxidisers in many soil environments globally, these findings advance significantly our understanding of terrestrial nitrogen cycling.

O NON 3

Persistence of the dominant soil phylum *Acidobacteria* by trace gas scavenging

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While it is well-appreciated that the vast majority of global soil bacteria exist in dormant states, the metabolic processes that enable them to survive fluctuations in energy availability remain to be elucidated. We will demonstrate that energy-starved cultures of *Pyrinomonas methylaliphatogenes* - an aerobic heterotrophic *Acidobacteria* isolated from New Zealand volcanic soils - persist by scavenging the trace concentrations of H_2 found in the atmosphere. The bacterium upregulates the expression of an eight-gene operon encoding a high-affinity hydrogenase following the transition from growth to persistence. A combination of activity assays show that the organism consumes H_2 using a membrane-associated, oxygen-tolerant hydrogenase. In addition to enhancing understanding of the biogeochemical hydrogen cycle and the second most dominant soil phylum, these findings suggest that scavenging of trace gases represents a widespread mechanism for the survival of soil bacteria.

O NON 4

Tropical soil-borne microbiome: linking diversity to function

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Soil microorganisms are sensitive to environment disturbances and such alterations have consequences on microbial diversity and functions. Our hypothesis is that alpha diversity of the microbial communities and functional diversity decrease from undisturbed to disturbed soils, with consequences for functional redundancy in the soil ecosystem. To test this hypothesis we used soil DNA shotgun metagenomics approach to assess the soil microbiome in a chronosequence of land-use from native tropical forest, followed by deforestation and cultivation of soybean croplands and pasture in different seasons. Agriculture and pasture soils were among the most diverse and presented higher functional redundancy, which is important to maintain the ecosystem functioning after the forest conversion. On the other hand, the ecosystem equilibrium in forest is maintained based on a lower alpha diversity but higher abundance of microorganisms. Our results indicate that land-use change alters the structure and composition of microbial communities, however the ecosystem functionality is overcome by different strategies based on the abundance and diversity of the communities.

Poster presentations

P SOIL 1

The Impact of Forest Logging and Oil Palm Plantations on Microbial Diversity and Activity in an Indonesian Tropical Peat Swamp Soil

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Indonesia contains the largest peat swamp area in Asia and the fourth largest in the world totalling 16.5 - 27 million hectares. Human activities (forest logging and agriculture) in Indonesian peat swamp forests are becoming a major concern in terms of the sustainability of the peatlands due to its essential services such as moderation of climate, energy fluxes and biodiversity conservation. The Giam Siak Kecil-Bukit Batu (GSKBB) tropical peat swamp forest landscape forms a Biosphere Reserve, listed as a World Heritage Forest Site. Unfortunately, up to 2009, some parts have been affected by illegal logging, while other parts continue to be affected by agricultural practices, primarily oil palm plantations. These practices resulted in significant ecosystem and ecological disturbance. In this project, soil samples of primary forest (pristine forest - PF), secondary forest (affected by illegal logging and regrown naturally - SF) and oil palm plantation with (OPP) and without burning (OPPB) from GSKBB sites were evaluated to assess microbial diversity and activity using molecular techniques (16S rDNA and Denaturing Gradient Gel Electrophoresis - DGGE) together with biochemical techniques (BIOLOG Ecoplate and enzyme activities). The results from SF, OPP and OPPB were compared with that from PF to examine the impact of human practices. PCR-DGGE analyses showed that these human practices changed the diversity of microbial communities (22-75% for bacteria, 17-36% for fungi and 7-18% for archaea). The results indicate that microbial phyla were significantly disturbed by soil perturbation and changing land use. The pattern of utilization of 31 carbon sources in Biolog Ecoplates showed reduced activities of bacterial community due to oil palm plantation practices; meanwhile logging practices on SF did not show any effect. In terms of soil enzyme activity, human practices increased β-glucosidase and cellobiohydrolase activities, but decreased chitinase and acid phosphatase activities. Overall, forest logging and agriculture practices in GSKBB biosphere reserve area were found to change the diversity and functioning of soil microbial community.

P SOIL 2

Proteomics reveals the impacts of deforestation on bacterial diversity and carbon fixation processes under arid climate

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The long-term effects of deforestation on the soil microbial community and its functionality are largely unknown. In order to assess simultaneously the phylogeny and functionality, we applied metaproteomics in soil samples from a natural area located in Southeast Spain, dominated by *Pinus halepensis* (F), and an adjacent area deforested 15-years ago (DF). Deforestation induced a long-term loss of bacterial biomass and microbial activity, but an increase in the bacterial diversity as estimated by metaproteomics. Protein abundances analysis revealed that *Proteobacteria* was higher in F than DF. In addition, a significant increase in the abundance of cyanobacterial proteins was observed in DF when compared to F. Interestingly, cyanobacterial proteins involved in carbon fixation (Ribulose 1,5-bisphosphate carboxylase, phycocyanins and photosystem proteins) were only identified in DF. The data suggest that *Cyanobacteria* play a critical role in the ecosystem functioning and biotic carbon fixation when soil is deforested in arid areas.

P SOIL 3

Beyond the extracellular-ecosystem: linking community variations, cellular functionality and phyla lifestyles of restored drylands

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The application of organic amendments in arid-degraded soils has been shown to benefit microbial mediated processes. However, despite the importance of soils for global sustainability, a gap has not been yet addressed in soil science: is there any connection between ecosystem-community processes, cellular functionality and microbial-lifestyles (i.e. oligotrophy-copiotrophy) in restored soils? Together with classical ecosystem indicators (fatty-acids, extracellular-enzyme activities, basal respiration), state-of-the-art metaproteomics was applied to fill this gap in a model-restoration experiment initiated 10-years ago by the addition of sewage-sludge and compost. Organic amendment strongly impacts ecosystem processes. Furthermore, the type of material used induces differences in the cellular functionalities through variations in the percentage of proteins involved in translation, transcription, energy production and C-fixation. We concluded that the long-term impact of organic restoration goes beyond ecosystem processes and affects cellular functionalities and phyla-lifestyles coupled with differences in microbial-community structures.

P SOIL 4

The cyanobacterial diversities of two different geothermal hot springs in Thailand

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The diversity of cyanobacteria was investigated in two different geothermal hot springs of Thailand. Soil samples were collected from San-kham-peang (Skp: at 18º 48' 49" N 99º 13' 46" E altitude 1,054 m; 78º C) and Nong-ya-plong (Nyp: at 13º 9' 11" N and 99º 36' 2" E altitude 1,003 m; 78º C). Cyanobacterial 16S rRNA gene were cloned from the extracted bacterial DNA and sequenced [1]. Bacterial phylogenic trees of these different geothermal regions were constructed and compared. Preliminary data collected from about 10% of 16S rRNA gene clone libraries showed that cyanobacteria from Nyp had a lower diversity in which only four species, *Chlorogloeopsis fritschii*, *Geitlerinema sp., Brasilonema octagenarum* and *Anabaena cylindrical*, were detected. Among ten species of cyanobacteria found in Skp, *Calothrix sp*. PCC 7507 was the dominant species. *Chlorogloeopsis fritschii*, the most abundant microbe in Nyp, was the only cyanobacteria that was detected in both places. The community of cyanobacteria from Nyp hot spring, Thailand was documented for the first time herein. Chemical composition analysis and the complete examination of clone libraries from both hot springs are in progress. Finally, two abundance-based estimators will be calculated to establish whether the libraries are large enough to yield stable phylotype richness estimates [2].

References

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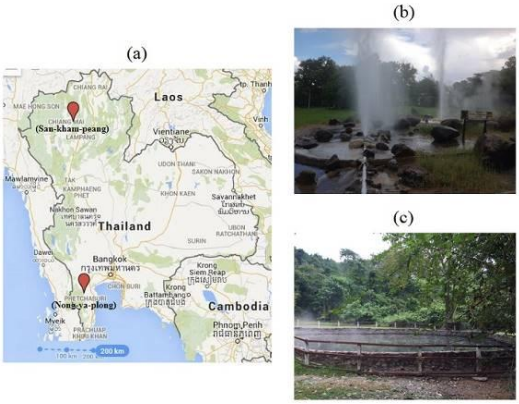
Figure 1. Localization of the geothermal springs of Thailand reported here (a) and overview of the hot springs San-kham-peang (b) and Nong-ya-plong (c).

Figure 1



P SOIL 5

Purification and identification of an antimicrobial and antitumoral agent isolated from *B. safensis.*

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Background: Here we present the Cyathin producing bacteria Bacillus safensis strain SG-32. This class of molecule has been isolated from various organisms, such as fungi and sponges, but there are no reports so far of this class of compounds being produced by bacteria. It is a diterpene member of the cyathane family, whose members are related to each other by a characteristic 5-6-7 tricarbocyclic fused-core structure. The molecule under study displayed an antibacterial and cytotoxic activity against human cancer cell lineages, but its most exciting therapeutic potential is derived from their ability to induce nerve growth factor release from glial cells, which implies in a therapeutic potential for the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson.
Objectives: Purification and identification of the B. safensis SG-32 extract with antimicrobial and anticancer properties.
Methods:Culture pellet and supernatant were obtained by successive centrifugation of culture medium after bacterial cultivation at 37oC for 72 h Cell biomass from pellet was submitted to soxhlet extraction system and supernatant extract was obtained by a liquid-liquid partition with a separatory funnel filled with culture and 500 ml of solvent. Three different solvents of different polarities were used: hexane, ethyl acetate and methanol. Purification was performed with chromatographic analyses in a glass column filled with silica gel. Fifty mL fractions were collected, which, after being analyzed by thin layer chromatography (TLC), were combined by similar compounds. The identification of the active compound, previously determined by bioautographic assay, was made by Nuclear Magnetic Resonance (NMR).
Results: The comparison of the experimental data obtained in this study with those of the literature (Shibata et al., 1998; Wachter et al., 1999; Urzúa et al., 2008; Enquist et al., 2009; Ayer et al. 1978,1979; Kamo et al., 2004; Kenmoku et al., 2002; Shiono et al., 2008; Marcotullio et al., 2006; Nicoletti et al., 1996; Dong et al., 2009; Ward et al., 1987) suggests that the compound obtained in fraction 3 of the cultivation extract of Bacillus safensis SG-32 is a diterpene with basic skeleton of Cyathin type. Full elucidation of the molecule has not been achieved yet based solely on NMR results. New experiments will be done in an effort to fully elucidate the structure of the molecule.

P SOIL 6

Phylogenetic analysis of antimicrobial-producing Actinomycetes isolated from Dry Dipterocarp Forest Soil in Northeast Thailand

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Thailand is located in the humid climatic area where a variety of tropical ecosystems are found. The diversity of organisms in Thai soil has been studies for decades, however, biodiversity of soil microorganisms in many areas of Thailand including Nakhon Ratchasima province remains uninvestigated and documented. This study has focused on the investigation of antimicrobial-producing Actinomycetes isolated from Dry Dipterocarp Forest soil at Suranaree University of Technology, Nakhon Ratchasima, Thailand (14.8729° N, 102.0237° E).
The 12 isolates of antimicrobial-producing Actinomycetes were obtained from 37 soil samples collecting during January 2012 to February 2014. Based on 16s rRNA genes analysis, these strains were close affiliated with the genus *Streptomyces* (11 isolates) and *Nonomuraea* (1 isolate). They could be subdivided into five different phylogenetically clusters. Most of isolates inhibited the growth of both Gram-positive bacteria and yeast, others were active against either Gram-negative bacteria or yeast. Only two isolates (PJ36 and PJ95) showed broad spectrum antimicrobial activities against Gram-positive bacteria, Gram-negative bacteria and yeasts. The 16s rDNA sequence of PJ36 and PJ95 showed 99% and 100% similarity with *Streptomyces chrestomyceticus* and *Streptomyces luteosporeus*, respectively. To our best knowledge, this is the first time an antifungal of *Streptomyces chrestomyceticus* (1) and *Streptomyces luteosporeus* are reported. The ability to produce antimicrobial substances of these strains might be an important trait for their successful colonization in soil.

Reference

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Figure legends
Figure 1. Dry Dipterocarp Forest at Suranaree University of Technology, NakhonRatchasima, Thailand
Figure 2. Phylogenetic affiliation of soil isolates and their antimicrobial activities against opportunistic pathogens
S. aur is *Staphylococcus aureus*; *S. epi* is *Stapylococcus epidermidis*; *B. sub* is *Bacillus subtilis*; *E. col* is *Escherichia coli*; *E. aer* is *Enterobacter aerogenes*; *P. aer* is *Pseudomonas aeruginosa*; *P. mir* is *Proteus mirabilis*; *S. mar* is *Serratia* marcescens; *C. alb* is *Candida* albicans; *C. tro* is *Candida tropicalis*; *S. cer* is *Saccharomyces cerevisiae*

Figure 1

Figure 2

Isolate name	Phylogenetic relationship		Opportunistic pathogens										
	Strain	% Identity	*S. aur*	*S. epi*	*B. sub*	*E. col*	*E. aer*	*P. aer*	*P. mir*	*S. mar*	*C. alb*	*C. tro*	*S. cer*
PJ33	*Streptomyces alboniger*	99	-	-	-	-	-	+	-	-	-	-	-
PJ36	*Streptomyces chrestomyceticus*	99	+	+	+	+	+	-	+	+	+	+	+
PJ43	*Streptomyces cinereospinus*	99	+	+	+	-	-	-	-	-	+	-	+
PJ51	*Nonomuraea jabiensis*	99	-	+	+	-	-	-	-	-	-	-	+
PJ75	*Streptomyces cyaneus*	99	+	+	+	-	-	-	-	-	+	-	+
PJ76	*Streptomyces iakyrus*	100	-	-	-	-	-	-	-	-	+	+	+
PJ77	*Streptomyces cellulosflavus*	99	+	-	+	-	-	-	-	-	-	-	+
PJ85	*Streptomyces durhamensis*	98	+	+	+	-	-	-	-	-	+	-	+
PJ88	*Streptomyces griseocarneus*	99	+	+	+	-	-	-	-	-	+	-	+
PJ90	*Streptomyces filipinensis*	99	+	+	+	-	-	-	-	-	+	+	+
PJ95	*Streptomyces luteosporeus*	100	+	+	+	+	+	-	+	-	+	+	+
PJ107	*Streptomyces filipinensis*	99	+	+	+	-	-	-	-	-	+	-	+

+ indicates inhibition; – indicates no effect

P SOIL 7

Zinc-lead mine soils shape genetic diversity and distribution of *Anthyllis vulneraria* rhizobial symbionts

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Introduction: Soils in mining areas enriched with heavy metals are a major cause of environmental pollutions. To limit metal pollution, ecological rehabilitation strategies are developed based on rare plant species able to grow on such soils. *Anthyllis vulneraria* was identified as an interesting metallicolous legume species growing on Les Avinières mine, and found efficiently associated to *Mesorhizobium metallidurans,* a nitrogen-fixing symbiotic species displaying high tolerance to Zn and Cd.
Objectives: This study is aimed to understand the distribution of the *M. metallidurans* populations as symbionts of *A. vulneraria*, in relation to a heavy metal content gradient in soils, because of their important implication for biological nitrogen entrance sustaining both mixed vegetation cover and soil quality recovery.
Materials & methods: Rhizobia were isolated from eight sites: four distant Zn-Pb mines, two moderately contaminated soils and two unpolluted soils, using *A. vulneraria* as trap plant. 116 strains were tested for their tolerance to Zn or Cd according to the Minimal Inhibitory Concentrations (MIC) measures. The rhizobial genetic structure was analyzed using taxonomic markers, a symbiotic marker (*nod*A) and a metal-tolerance marker (*cad*A).
Results: The distribution of *M. metallidurans* was strictly related to a high contamination of soils with heavy metals. The rhizobial diversity in unpolluted sites revealed the dominance of new *Mesorhizobium* species, all are metal-sensitive and distinctly separated from *M. metallidurans*. Moderately contaminated soils shows either a population structure similar to those of unpolluted sites, or another pattern of diversity with a mixture of sensitive strains phylogenetically distinct from strains of unpolluted sites and metal-tolerant ones belonging the *M. metallidurans* species. Symbiotic diversity reflected by a nodulation gene (*nod*A) reveals two symbiovars that did not correlate with taxonomic clustering and which were related to geographical sites whatever their metal contents.
Conclusion: Metal contents in mining soils shape *Anthyllis* rhizobial composition with specific metal-tolerance and taxonomic diversity. By contrast there was no effect of the metal content in the soil on the symbiotic diversity.

P SOIL 8

Towards net zero/negative methane emission in agricultural soils: Unexpected atmospheric methane consumption after amendments with specific residues.

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The growing human population and scarcity of arable land necessitate agricultural intensification to meet the global food demand. Intensification of agricultural land entails residue inputs into agro-systems, however, these may cause increased greenhouse gas emissions (CH_4, CO_2, and N_2O). In particular, anomalies in atmospheric CH_4 concentrations, including the recent CH_4 increase are a cause for global concern as CH_4 has a 34 times higher global warming potential than CO_2 in a 20-year scale. We tested a range of bio-based residues with a broad spectrum of properties (e.g. C/N ratio, recalcitrance) to determine their impacts on greenhouse gas emission with focus on CH_4 in two representative agricultural soils (i.e. clay and sandy loam). Potted incubations containing soils amended with and without residues were performed over two months. Unexpectedly, we detected transient CH_4 consumption after incubation with specific residues in both soils. Further batch incubations confirmed significantly higher CH_4 oxidation rate in these soils after residue amendments, demonstrating that CH_4 oxidation was induced by residue addition. A diagnostic microarray analysis of the *pmoA* gene specific for the methanotrophs revealed the presence of a relatively low diverse community comprising of only Alphaproteobacterial methanotrophs, suggesting their role as CH_4 sink in agricultural soils. A specific quantitative PCR assay will be performed to detect changes in the gene abundance of this methanotrophic sub-group. Furthermore, considering CH_4 production rate as the functional response variable, we determined the relative contribution of residue (manure)-derived and soil-borne methanogens to total CH_4 production. Results show that only the soil-borne methanogenic community was significantly stimulated by manure amendment, resulting in increased CH_4 production. Overall, our results demonstrated that with a smart choice of residues, agricultural soils commonly considered to be a CH_4 source, can become a CH_4 sink. Nevertheless, field studies are needed to support the current findings. Our results also stress the importance of the indigenous soil microbial communities and physiochemical properties of a residue when considering CH_4 mitigation strategies in agricultural lands.

P SOIL 9

Microbial community structure doesn't matter in litter decomposition.

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Mixed tree plantations have been studied due their potential to improve biomass production, ecosystem diversity and soil quality compared to monoculture systems. One example is the mixture of *Eucalyptus* and *Acacia* trees, which has been showed as a promising strategy to improve microbial diversity and nutrient cycling in soil. In this current study we show how the mixture of these species can change the microbial community associated with litter and what is the impact of the microbial community and the environment over the litter decomposition. We studied both bacteria and fungal community associated with litter from pure and mixed plantations using next generation sequencing. We evaluate the effect of both plant material and local of incubation over the microbial community and decomposition rate, using litterbags incubated *in situ*. We discover that there is significant influence of both plant material and local of incubation in the litter decomposition. The plant material is the main modulator of the microbial community with very distinct microbial communities between the plant species, and the litter of mixed plantations showed an integration of the microbial community found in each species separately. The community change very little in function of time, and the local of incubation does not influence the microbial community. The soil and the litter do not share the microbial community. Surprisingly, the microbial community structure and diversity does not have any association with the speed of decomposition, and the differences in the decomposition patter are explained basically in function of the nitrogen compounds between the materials. Still, our results indicate that the microbial community responsible for litter decomposition probably is establish before the leaves fall. We hypothesized that microbial community responsible for litter decomposition probably is establish before the leaves fall.

P SOIL 10

Removal of Geosmin by a bacterium isolated from biological activated carbon

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Question: Recently, production of taste and odour (T&O) compounds is a common problem in water industry. Geosmin and 2-Methylisoborneol (2-MIB) are the two musty odour components in drinking water. It have been reported that T&O have psychosomatic effects that cause consumer complaints, such as headache, stress, stomach upset. Nevertheless, T&O compounds are hardly eliminated through the conventional water treatment systems, among which an adsorption process called biological activated carbon (BAC) treatment process is the most commonly used process. Microorganisms on the BAC can degrade ammonium nitrogen, dissolved organic matter, and T&O compounds regenerating clean water.
Methods: In this study, we examined removal rate of Geosmin by a bacteria having Geosmin-degrading properties isolated from a BAC process in a water treatment plant. The Geosmin-degrading gene and 16S ribosomal RNA gene were purified from the bacteria using conventional microbiological methods, and then used for PCR amplification. Identification of the bacteria was confirmed by analyzing sequence of the 16S rRNA gene using the Basic Local Alignment Tool (BLAST) supported by National Center for Biotechnology Information (NCBI). Geosmin in mineral salts medium (MSM), with initial dose of 0.01mg/L, was used to investigate the biodegradation of geosmin as the sole carbon source. Geosmin removal rates were analyzed by a gas chromatography equipped with a mass selective detector coupled to a solid phase microextraction.
Results: Total 8 strains of bacteria were isolated from the BAC: these were identified to be *Chryseobacterium* sp., *Bacillus megaterium*, *Enterobacter cloacae*, *Aeromonas hydrophila, Serratia* sp., *Micrococcus luteus*, *Stenotrophomonas* sp., *Aeromonas* sp. Removal rates of 0.01mg/L Geosmin in MSM by the bacteria were 25%, 0%, 4%, 11%, 22%, 14%, 18% and 36% for *Chryseobacterium* sp., *Bacillus megaterium, Enterobacter cloacae*, *Aeromonas hydrophila, Serratia* sp., *Micrococcus luteus*, *Stenotrophomonas* sp. and *Aeromonas* sp., respectively. These results indicate that the bacterial strains in BAC treatment process were investigated by characterizing bacterial 16S rRNA gene sequence analysis.
Conclusions: Herein, we found 8 strains of bacteria were identified and some bacteria could play an important role in the adsorption and biodegradation of Geosmin. Classification of the microorganisms at various (waste) water plant of BAC process rapidly with higher accuracy and Geosmin removal efficiency are our next goal.

P SOIL 11

Analysis of attached bacterial communities during biological activated carbon process in drinking water treatment plants

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Question: Currently, a common problem in water industry is the production of taste and odour compounds (T&O). Geosmin and 2-Methylisoborneol (2-MIB) are one of the musty odour components occurrence of which in water is a matter of great interest. Among the advanced treatment process, adsorption process such as biological activated carbon (BAC) is most commonly used for this problem. Thus, the identification of microorganisms community of BAC is important to predict the BAC capacity to remove T&O.
Methods: BAC was collected from the Seongnam, Goyang drinking water treatment plant and Goryeong pilot plant. Detached bacteria were serially diluted in 0.85% NaCl solution and 100 µL of each suspension was spread on a R2A agar and incubated at 37°C for 5 days. After incubation, colony forming units (CFU) in the planted drops, containing 30 to 300cells/g were counted and the original number of CFU was calculated. The DNA from the BAC samples was used as template for PCR amplification of the 16s rRNA gene. Identification of the bacteria was confirmed by analyzing sequence of the 16S rRNA gene using the Basic Local Alignment Tool (BLAST) supported by National Center for Biotechnology Information (NCBI).
Results: The heterotrophic plate count (HPC) was ranged from $3x10^5$ to $4x10^5$ CFU/g, $2x10^6$ CFU/g, $7x10^5$ CFU/g, respectively from the Seongnam, Goyang drinking water treatment plant and Goryeong pilot plant, respectively. Three bacteria species including *Hydrocarboniphaga effuse*, *Bacillus cereus*, *Cupriavidus metallidurans* were dominantly detected from Seongnam drinking water treatment plant, two bacteria species including *Novosphingobium rosa*, *Sphingomonas*

sanxanigenens were dominantly detected from Goyang drinking water treatment plant and two bacteria species including *Devosia insulae*, *Afipia broomeae* were dominantly detected from Goryeong pilot plant.
Conclusions: The biomass and bacterial communities in the BAC samples collected from different drinking water treatment plants may influenced by size pore, surface structure of activated carbon and temperature, pressure, flow rate, process periods and qualities of raw water. Therefore it is necessary to monitor the attached bacteria periodically. We will further study the distribution of bacterial communities isolated from BAC and investigate whether these bacteria are capable of degrading T&O compounds.

P SOIL 12
Bacterial community responses in mixed forestry plantation of *Eucalyptus* and *Acacia*.
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Bacteria are important components of soil, being key organisms in nutrient cycling and plant health. In long-term plantations, such as forestry, the interactions between plant and soil microorganisms are of great importance, since they establish mutualistic relationships along time, which will reflect in ecosystem productivity. In this context, we were interested in understanding how soil bacteria respond to two different tree species (*Eucalyptus urograndis* and *Acacia mangium*) cultivated in pure (100% of one specie) and in mixed stands (50:50 of each specie interleaved). Samples were collected in two consecutive years (plants with two and three years old) from an experimental field in quadruplicate. Soil properties and bacterial community were analyzed. The data showed no significant difference of the diversity indexes among treatments, but a significant increase of bacterial diversity from the second to the third year in all of them was observed. Eighteen different phyla were found with high predominance of Firmicutes, Proteobacteria, Actinobacteria and Acidobacteria. While Proteobacteria was the most abundant in *Eucalyptus* plantation, Firmicutes was more abundant in *Acacia* and in the mixed stands. The genus Bacillus was highly abundant in all samples, with up to 22% of all sequences. A significant treatment effect was revealed by the NMS ordination of the shared OTU data, with a small difference between the two pure stands. The functional inference of the community showed a significant and more pronounced change in the functional profile of the community among treatments, with a higher influence of *Acacia* compared to the *Eucalyptus* in the mixed stands. The changes in the relative abundance of some groups reflected in the soil functional profile. Compared to a study of the soil fungal community under the same treatments, bacteria was less sensitive to treatments, and there was no correlation between bacterial and fungal communities.

P SOIL 13
Assessing the composition of bacterial community associated with different types of deadwood
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The decomposition of deadwood substantially contributes to the carbon cycle and therefore is one of the key processes in temperate forests. This process is driven by saprotrophic organisms such as fungi and bacteria. While the role of fungi in deadwood decomposition was repeatedly addressed, there are just a few surveys of deadwood-associated bacteria focused on the initial stage of decay. The colonization of deadwood by bacteria is influenced by various factors such as microclimate conditions, tree species and volume. The aim of this study was to describe the composition of bacterial community in the initial stage of *Fagus sylvatica* and *Abies alba* decomposition of fine and coarse woody debris in the Bavarian Forest National Park, Germany. Samples were collected after one and two years of decomposition by drilling logs (mean diameter 30 cm) and branches (5 cm). The composition of bacterial community was characterized by 16S rRNA sequencing on the Illumina MiSeq platform. Two sets of samples with interval of one year between each other allowed comparison of community composition on time scale. Members of the classes *α-*, *β-*, and *γ-Proteobacteria* and the phyla *Actinobacteria*, *Bacteroidetes* and *Acidobacteria* were dominant in all samples. Bacterial community composition was affected mainly by diameter of wood. The effect of tree species was lower but still significant. This is in contrast to the results of fungal community analysis where tree species was a better predictor of community composition. The genera *Sphingomonas*,

Mucilaginibacter, *Burkholderia* and *Pedobacter* which were previously described as cellulolytic were detected as abundant. Also the genus *Streptomyces* described as ligninolytic was observed. Higher values of enzyme activities were recorded in deadwood with lower diameter (branches). This can correlate with higher relative abundances of some mentioned genera capable of wood decomposition in branches. Presented study shows that the composition of bacterial communities associated with deadwood in the initial stages of decomposition is driven by other factors that this of the fungi which quantitatively dominate the decomposing wood.

P SOIL 14

Multi-analytical approach reveals potential microbial indicators in soil for sustainable sugarcane model systems

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This study focused on the effects of organic and inorganic amendments and straw retention on the microbial biomass (MB) and taxonomic groups of bacteria in sugarcane-cultivated soils in a greenhouse mesocosm experiment monitored for gas emissions and chemical factors. The experiment consisted of combinations of synthetic nitrogen (N), *vinasse* (V; a liquid waste from ethanol production), and sugarcane-straw blankets. Increases in CO_2-C and N_2O-N emissions were identified shortly after the addition of both N and V to the soils, thus increasing MB nitrogen (MB-N) and decreasing MB carbon (MB-C) in the N+V-amended soils and producing chemical characteristics that were correlated with the MB. Across 57 soil metagenomic datasets, *Actinobacteria* (31.5%), *Planctomycetes* (12.3%), *Deltaproteobacteria* (12.3%), *Alphaproteobacteria* (12.0%) and *Betaproteobacteria* (11.1%) were the most dominant bacterial groups during the experiment. Differences in relative abundance of metagenomic sequences were mainly revealed for *Acidobacteria*, *Actinobacteria* and *Verrucomicrobia* with regard to N+V fertilization and straw retention. *Actinobacteria* were more responsive to straw retention with *Rubrobacterales*, *Bifidobacteriales* and *Actinomycetales* more closely related to the chemical characteristics of N+V-amended soils. *Acidobacteria* subgroup 7 and *Opitutae*, a verrucomicrobial class, were closely related to the chemical characteristics of soils without straw retention as a surface blanket. Differential abundances in bacterial groups were confirmed using 16S rRNA gene-targeted phylum-specific primers for real-time PCR analysis in all soil samples. Taken together, the results showed that MB-C and MB-N responded to changes in chemical characteristics and CO_2-C and N_2O-N emissions, especially for N+V-amended soils. The results also indicated that several taxonomic groups of bacteria, such as *Acidobacteria*, *Actinobacteria* and *Verrucomicrobia*, and their subgroups acted as early-warning indicators of N+V amendments and straw retention in sugarcane-cultivated soils, which can alter the soil chemical characteristics.

P SOIL 15

Effects of biochar addition on bacterial populations of the lettuce and strawberry rhizosphere

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The rhizosphere is the narrow zone around the plant roots. It consists of a complex root-associated microbiome, containing over 10^9 microbial species per gram of soil, and is crucial for plant growth and health. We have investigated the effects of soil amendments on the rhizosphere microbiology of lettuce and strawberry. In the presented research, biochar, the solid coproduct of biomass pyrolysis, is used as a soil amendment. Biochar has potential to help mitigate climate change and may be beneficial for the crop.
Lettuce and strawberry plants were grown for 2-3 months in respectively field soil and peat, each amended with 0, 1 or 3% (dry weight) biochar produced from holm oak at 650°C for 12-18h in the frame of the FP7-Fertiplus project

(www.fertiplus.eu). 16S (V3-V4) amplicon sequencing was used to study the bacterial composition of the lettuce and strawberry rhizosphere, and possible shifts induced by the addition of biochar.
No significant effects of biochar addition on the lettuce rhizosphere were observed. In contrast, incorporation of 3% biochar induced significant changes in the strawberry rhizosphere microbiome. A significant reduction in relative abundance of Proteobacteria was measured, mainly due to a reduction in *Burkholderia* species. Moreover, this mix also enhanced bacterial richness and diversity. This microbial information was combined with plant and substrate properties, showing a relation between biochar addition and strawberry plant growth, resistance to *Botrytis cinerea*, substrate moisture content and water-soluble phosphorus.
In conclusion, our data showed that biochar incorporation in peat induces changes in the bacterial rhizosphere community of strawberry. This bacterial shift corresponded to changes in substrate properties, plant growth and plant resistance.

P SOIL 16

Plant bacterial inoculants as invaders on crop fields: Invasion ecology applied to rhizosphere microbiomes

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The use of bacterial inoculants to improve crop productivity reduces the need for fertilizer and other agrochemicals without reduction on grain yield. These inoculants, however, might not be always effective under field conditions. As interaction between the inoculants and the native microbial community plays a key role in effective root colonization and plant growth promotion, classical invasion ecology might be applied for inoculants on crops to predict results and improve microbial management effectiveness. In this work, we apply hypothesis based on invasion ecology to crop fields under bacterial inoculation. We evaluate the associated microbial community composition of inoculated and non-inoculated plants to test if: (I) an inoculant with a higher invasion potential will provide a higher plant growth promotion effect; (II) if lower diversity/resource ratio in the environment facilitates invasion. To do this, we analyzed 3 different maize crop locations in south Brazil, which received the same 4 bacterial inoculants (one *Azospirillum,* one *Achromobacter,* and two *Pseudomonas*). Samples were taken from bulk soil immediately before planting, and from rhizospheric soil of 10 days old plants. Metagenomic DNA was extracted, the V4 region of the 16S rDNA gene was amplified, amplicons were sequenced on the MiSeq platform, and sequences were processed using QIIME. OTU frequency data was used on PCoA and SIMPER tests. All SIMPER results were then ploted on a PCA, showing how dissimilar were the differences in the control-treatment pairs across the locations, and this approach was used as a proxy for inoculant invasion ability. PCoA has shown that samples clustered by the 3 locations, but the PCA based on SIMPER results suggested that 2 different inoculants might induce very different community changes depending on crop field location. These larger differences that would indicate greater invasion ability of the inoculant, however, were not associated to greater crop productivity. The diversity/resource ratios are still to be calculated, so invasion difficulty across environments must yet be estimated. Our preliminary conclusion is that it is not possible to predict the most effective inoculants by looking at shifts on the rhizospheric community 10 days after planting - what would be extremely useful in field trials.

P SOIL 18

A functional view of mangrove microbiomes trhough meta-omics

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Microbial life is a key component of mangroves soils that has been extensively described but rarely targeted by function-based approaches. Here we applied a combination of metagenomics and metatranscriptomics libraries to infer on microbial communities across four mangroves under distinct stage of preservation, located along the coastline of the State of São Paulo (Brazil). Sequences resulted from DNA or RNA (total and mRNA enriched) high throughput sequencing was developed and evaluated using MG-RAST to describe the taxonomy and function of the microbial groups distributed among mangrove areas. The first insights confirmed that the main groups in both, DNA and RNA-based, had a higher abundance of Deltaproteobacteria, Alphaproteobacteria and Gammaproteobacteria. In exception, the oil-contaminated areas, presented an increasing frequency of Betaproteobacteria and Epsilonproteobacteria in the RNA. A comparative analysis of DNA or RNA revealed similar patterns, suggesting that there is a microbe turnover in the mangroves. Sequences affiliated to functions involved in nitrogen and sulfur cycling was found in similar frequencies in each mangrove (DNA and RNA-based analyses).

In overall, these findings demonstrated that distinct microbial groups play crucial roles in these environments. These results suggest that microbial diversity, on the basis of its functional redundancy, is able overcome impacts on these biogeochemical cycles caused by environmental contamination. In combination, the results foster our knowledge on the taxonomical and functional groups of microbes in mangroves soils, reinforcing the utility of coupled DNA and RNA analysis for describing the microbiome communities in soils.

P SOIL 19

Isolation and characterization of an endophytic bacterium, Bacillus megaterium BMN1, associated with root-nodules of Medicago sativa L. growing in Al-Ahsaa region, Saudi Arabia

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Introduction: alfalfa represent 64% of the total area devoted to fodder cultivated area in Saudi. Rhizobacteria have roles in sustaining agriculture via nitrogen fixation and plant-growth promoting activities . Reports on bacterial endophytes of alfalfa nodules are few.
Objectives: Characterization of endophytic bacteria from root-nodules of Alfalfa and assessment their effects on growth of three economically-important crop legumes.
Materials and Methods: Endophytic bacteria were isolated on yeast-extract mannitol agar, identified using 16S rDNA sequencing and characterized phenotypically using API20E and Ch50 strips, IAA production and solubilize inorganic phosphate . The effects of the strains on *Lens esculentus*, *Phaseolus vulgaris* and *Pisum sativum* were assessed.
Results: Sixty-five strains were obtained and utilized 50% of the different chemical substrates contained in the API20E strip and API50CH. Interestingly, 65% of the strains produced acetoin, which plays an important role in induced systemic resistance. Twenty five strains IAA and solubilize inorganic phosphate. Few strains exhibited antibacterial or antifungal activities. All the strains had positive effects on one or more of the growth parameters (dry weights of roots, shoots and nodules) for tested plants. The strains were identified using 16srDNA sequencing as *Enterobacter cloacae*, *Bacillus megaterium, Bacillus* spp., *Staphylococcus aureus* and *Sinorhizobium meliloti*.
Conclusion: The root-nodules Alfalfa harbored diverse bacteria that are interacting in one way or another to improve plant growth via direct or indirect mechanisms. The strains belonged to plant growth promoting rhizobacteria and could have significant agricultural applications to increase plant productivity and reduce the use of synthetic fertilizers and pesticides.

Figure 1

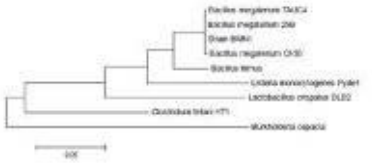

FIG. 2 – Maximum Likelihood phylogenetic tree based on 16S rDNA gene sequences showing relationships between strain BMN1 (accession no. KJ461522) and related species *Bacillus megaterium* strain 29B (accession no. KC329822.1), *Bacillus megaterium* strain C5-S5 (accession no. KC329822.1), *Bacillus megaterium* strain TAUC4 (accession no. HQ914779.1), *Bacillus firmus* (accession no. FJ138300). In addition, representatives from the Firmicutes; *Listeria monocytogenes* strain Pydel (accession no. KC852899), *Lactobacillus crispatus* strain BL02 (accession no. AF243141) and *Clostridium tetani* strain ET1 (accession no. DQ978212) and *Burkholderia cepacia* (accession 366 no. U96027.1) were also included.

P SOIL 20

Bacterial and archaeal diversities in San Kamphaeng hot spring, Chiangmai, Thailand

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The bacterial and archaeal diversities of soil in the hot spring from San Kamphaeng, Chiangmai, Thailand were investigated. The range of the soil temperature was 50-60 °C. To study the microbial communities, the DNA was extracted from the brown sandy-soil and 16S rRNA was amplified. Approximately 5% of the clone library was picked up for this preliminary study. The data from DNA sequencing were analysed and the phylogenetic analysis showed that eubacteria was more diverse than archaea. Thirty species of bacteria were detected and seven of them were cyanobacteria. All of cyanobacterial species found in this study have not been reported in the previous study [1]. *Calothrix sp.* was the dominant specie of cyanobacteria whereas *Thioflavicoccus mobilis* was dominant in the bacterial species. Twelve species of archaea were also found. Among these archaeal species, *Methanococcus aeolicus* was found dominantly in the area. To our best knowledge, this is the first report about archeal diversity of San Kamphaeng hot spring.

Reference

1. Sompong U, Anuntalabhochai S, Cutler RW, Castenholz RW, Peerapornpisal Y: **Morphological and phylogenic diversity of cyanobacterial populations in six hot springs of Thailand**. *Scienceasia* 2008, **34**(2):153-162.

Fig.1 The localisation (A) of the hot spring (B) in San Kamphaeng, Chiangmai, Thailand

Figure 1

P SOIL 21

Effect of different organic amendments on soil microbial communities

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The use in agriculture of organic fertilizers of residual origin make possible to increase productivity with lower cost, while side effects on soil biology are less known. Yet, soil biological communities are of major importance for the delivery of many soil ecosystem services. The objective of the present study was to assess the effect of organic amendments (OA) on the diversity and taxonomic composition of soil bacterial and fungal communities using 454-pyrosequencing the 16S-Bacterial and 18S-Fungal ribosomal genes.

Two experimental sites were used with different rates of application every 2 years: Feucherolles (VERI-INRA collaboration, 4 t C/ha) and Colmar (0.84 to 2.2 t C/ha). Both sites received three different OA compared to a control treatment (TEM): a biowaste compost (BIO), a farmyard manure (FYM), and a compost issued from the co-composting of green wastes with sewage sludge (GWS). For each site, common amendments had same or different origins. Specifically, Feucherolles

received a municipal solid waste compost (MSW); and Colmar a non-composted sewage sludge (SLU) and a composted farmyard manure (FYMc). Soils sampling was achieved one year after the application of OA.
OA inputs changed the structure of bacterial and fungal communities. For Feucherolles, no effect of OA was observed on the structure of bacterial community. Concerning the fungal community, BIO inputs had the highest impact on the structure compared to the other OA with a decrease of *Basidiomycota* relative abundance. In Colmar, soil bacterial community structure was more sensitive to organic amendment. Indeed, compared to the control, FYM inputs induced an increase of *Crenarchaeota* and a decrease of *Thaumarchaeota*. FYMc led to a decrease of *Thaumarchaeota* and *Acidobacteria*, while SLU carried to a decrease of *Chloroflexi*. Fungal composition was also impacted by the organic product amended, especially by the FYMc one, where an increase of *Chytridiomycota* was recorded.
These results highlight that OA have an effect on soil microbial community diversity, which depends on the quality of the amendment and the field site. More studies are now needed to assess how this links to soil functioning and to determine to what extent the effect of OA on soil microbial communities may be modulated by the pedoclimatic soil properties.

P SOIL 22
Changes in Cerrado biome microbial communities' composition and function in response to seasonal variations in water availability.
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Introduction: The Cerrado is the largest tropical savanna in the world and it occurs primarily in Brazil. Soils in this biome are highly weathered, with high clay content, low pH and high iron levels. There are two defined seasons, a wet rainy summer and a very dry winter.
Objectives: This worked aimed at determining the microbial assembly variations, taxonomically and functionally, according to changes in water availability during the wet and dry seasons in soils from savanna-like.
Materials and Methods: Using barcode pyrosequencing of the rRNA genes and shotgun metagenomics analysis, a total of the 4 pyrosequencing runs were performed with DNA directly extracted from four contrasting Cerrado vegetation types ('*Cerrado denso*', '*Campo sujo*', '*Cerrado sensu stricto*' and Galery forest) in the two different seasons (drought and rain).
Results: Changes in bacterial, archaeal, and fungal community structures in the cerrado denso, cerrado sensu stricto, campo sujo, and gallery forest soils strongly correlated with seasonal patterns of water availability. There was a strong influence of different levels of water availability in the taxon composition of all communities analyzed. The relative abundances of *Chloroflexi, AD3*, *Acidobacteria subgroup-6*, *Basideomycota* and *Ascomycota* were negatively correlated with temporal variations of soil´s moisture under different plant vegetation types; in contrast, *Alpha/Gammaproteobacteria, Gemmatimonadetes, Bacteroidetes* and *Crenarchaeota* showed an increase in their relative abundances as the moisture levels in soils increased ($P < 0.01$ in all cases). In addition, shotgun metagenomic analysis revealed an increase in the relative abundance of genes associated with iron acquisition, transposable elements, dormancy and sporulation in the dry season.
Conclusions: This change in the repertoire of genes may reflect a functional response to environmental stress, as microorganisms adapt to an environment with decreasing water availability. These results may help answer questions about how interactions between different vegetation types and precipitation regimes might collectively alter abundance, structure and functions of microbial community in this particular ecosystem.

P SOIL 23
Temperature effects on recovery time of bacterial growth after rewetting of dry soil
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Drying/rewetting events have been identified as a global change issue with both frequencies and duration of droughts being expected to change in the future. Rewetting dry soil will result in an immediate increase in respiration, while bacterial growth will recover at a slower pace. The recovery time may also be affected by other environmental factors, like temperature.

In a laboratory experiment the recovery of bacterial growth after rewetting was measured in a soil, where bacterial growth rates start to increase in linear fashion immediately after rewetting (type 1 response *sensu* Meisner et al., Soil Biol. Biochem. 66:188-192, 2013),. Air dried soil was rewetted at three temperatures (5, 15 and 25°C) by adding water to achieve 30% moisture. The bacterial growth over time was then estimated using the leucine incorporation method and the time to recover to growth rates in moist control soil calculated.
While the recovery time was only 6h at 25°C, it increased to 57h at 15°C and 175h at 5°C. Thus, a temperature difference of 20°C resulted in 30 times longer recovery times. The temperature dependency of the recovery time was well modeled by a square root function, similar to bacterial growth in soil. We conclude that the effects of environmental perturbations, like changes in soil moisture, will be modified by other environmental factors, like temperature. Temperature will thus not only have profound effects on soil functions, directly affecting bacterial growth rates, but also affect length of transition periods, like resuscitation after a drying event.

P SOIL 24

Bacterial enzyme systems for the decomposition of cellulose and hemicellulose contained in plant biomass from forest soil are complex and highly diverse

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Dead plant biomass is one of the major sources of energy for microorganisms inhabiting the forest soil and its decomposition in one of the key steps in the carbon cycle in these ecosystems. Evidences show that bacteria are taking active part in the degradation of cellulose and hemicellulose; however, the role of different bacterial taxa in decomposition of plant biomass is still unclear. New molecular methods are helping us to delve into the diversity of bacterial sets of cellulases and hemicellulases acting in this process, and to shed light on their different modes of action in plant biomass deconstruction. In this way, omics approaches based in the study of genomes and proteomes offer an interesting and vast source of information about the cellulolytic abilities of bacteria. This work was initiated by the screening and identification of bacterial strains showing potential cellulolytic activity from litter and organic soil of a temperate oak forest (Czech Republic, Central Europe). Four strains (*Mucilaginibacter* L294, *Pedobacter* O48, *Luteibacter* L214 and *Paenibacillus* O199) were whole-genome sequenced, putative protein-coding sequences were identified and CAZy domains involved in plant biomass degradation were annotated. Although the four genomes showed numerous CAZy families, the composition and number of GHs families encoding for potential cellulases and hemicellulases were highly different among the four isolates. Moreover, secretomes from bacteria growing independently in plant biomass and cellulose were analyzed with 1D PAGE-LC-MS/MS. This technique allowed us to link the gene pool from genome with protein expression. Results showed four different enzyme systems for cellulose degradation, involving different proteins from multiple GH and CBM families for each bacterial strain. Our data indicate high structural diversity existing in the decomposition process in forest soil, and confirm the role of the studied bacterial taxa as important cellulose decomposers in this ecosystem.

P SOIL 25

Accessing the microbiome of Brazilian sugarcane soils through metagenomics

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The sugarcane crop has a huge impact on Brazilian agriculture. A better understanding of the microbial composition in soils with sugarcane, especially the role that microorganisms involved in the nitrogen (N) cycle is of utmost importance for its cultivation productivity and sustainability. However, little is known about the relationship between N-related microbial communities and this crop. Here we targeted three areas used for sugarcane cultivation (named A, F and J), which present a range of different management conditions and soil characteristics. Each area has triplicate sampled, and soil DNA was used to shotgun metagenomics sequencing (SMS) by Illumina Hiseq2500 (average of 3.4 billion of bp per metagenome). The majority of SMS reads were derived from Bacteria (98% hits to M5NR database); but sequences also matched Eukarya (1%) and Archaea (1%). The three most abundant phyla related to Bacteria domain were *Proteobacteria* (44%), *Actinobacteria* (26%) and *Acidobacteria* (6%). For Archaea, reads matched with *Euryarchaeota* (33%), *Thaumarchaeota* (28%) and *Crenarchaeota* (20%). Levels of α-diversity (based on Shannon index) were higher for bacterial communities than archaeal communities presented in all 9 metagenomes. Therefore, we focused on N-related microbial communities, where similar

frequencies of reads were observed among most metagenomes. Functions like nitrogen fixation and ammonium oxidation were the most abundant (SEED database) and the bacterial and archaeal group present in greater amount responsible for carrying them out were *Proteobacteria* and *Thaumarchaeota*, respectively. The next step will be to understand the relationship between these microorganisms and the special characteristics of each area, thus contributing to clarify which type of management and physical/chemical parameters act on the abundance of them.

P SOIL 26

Concentration-Modulated Growth in Reduced Communities: Distinguishing Responses of Soil Microorganisms by Micro Segmented Flow

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Soil communities are highly dynamic system, which are modulated in their composition and activity by physical and chemical factors. It has to be assumed that soil contains a restricted number of active species, but a high number of species in a dormant state which are activated only in case of changing environmental conditions. New strategies and cultivation techniques are required for the evaluation of this ecological potential.

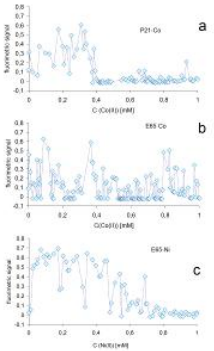
A new experimental approach has to address the fact that the fractal porous structure of soils represents a network of partially decoupled cultivation and reaction volumes. Reduced numbers of organisms and species are mainly interacting in these small volumes. The soil as whole represents a large set of these “reduced communities”. The technique of micro segmented flow can mimic this situation in laboratory screenings. It allows to subdivide soil samples into small fractions containing such “reduced communities” and to cultivate them under well-defined conditions in large sample sets. In particular, this technique is suited for the variation of concentrations of effector substances and for the determination of highly resolved dose/response functions.

The concentration-dependent response of natural soil microbial communities from ancient mining areas has been studied. Sets of about 200 droplets were incubated between 2 and 7 weeks and measured by micro photometry and micro fluorometry. In result, very different response patterns have been found: Sharp transitions between a concentration range with dominating strong growth and a flowing concentration range with strongly reduced growth were observed in many cases (example in Fig. 1a). This finding indicates changing growth conditions in very small concentration intervals and a switch-like response of the activity of involved organisms. In other cases, a stochastic response (example in Fig. 1 b) or a superposition of a stoachastic response and smoother transitions (example in Fig. 1c) have been found.

In conclusion, micro segmented flow is a very promising strategy for studying the ecological potential of soil microorganisms communities.

Figure
Examples of higly-resolved dose/response functions obtained from reduced communities of microorganisms from soil samples by concentration-dependent cultivation (21 days) in individual micro fluid segments (0.5 µL)

Figure 1



P SOIL 27

COMPARATIVE EVALUATION OF BACTERIAL DIVERSITY FROM GM AND NON-GM MAIZE RHIZOSPHERE

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The rhizosphere is a critical interface supporting the exchange of resources between plants and their associated soil environment. Rhizosphere microbial diversity is influenced by the physical and chemical properties of the rhizosphere, some of which are determined by the genetics of the host plant. However, within a plant species, the impact of genetic variation on the composition of the bacterial biota of GM and Non-GM maize rhizosphere is poorly understood. Here, we studied the bacterial diversity and population dynamics in the rhizosphere of one GM and two Non-GM maize cultivars using cultured and uncultured based analysis (16S rRNA gene-based molecular analysis). Using pyrosequencing of bacterial 16S rRNA genes, around 160,000 sequences were obtained (20,000 reads per sample) representing 21 phyla's, 184 families, 469 genera and a small amount of unclassified bacteria. Based on cultured and uncurled approaches no significant variation was observed in the transgenic samples as compared to un-transgenic.

P SOIL 28

Control of Boreal Forest Soil Decomposition Processes by Plant Secondary Compounds

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Introduction: Hundreds of thousands of different secondary plant metabolites (SPMEs) are produced by plants, however the quantity and variety of compounds vary enormously between plant species/progenies and shift in response to environmental conditions. We hypothesize that SPMEs released through litterfall and root turnover in the boreal forest control ecosystem carbon cycling by inhibiting microbial decomposition processes, which are overcome partially by increased aromatic biodegradation of microbial communities that also fortuitously prime soils for accelerated biodegradation of contaminants.
Objective: This study aims to reveal how SPMEs, released through litter deposition, exert control on cycling of complex organic matter (carbon and nutrients) in soil, and how these chemical controls are overcome by shifts in microbial communities that also affect resilience to contaminants in the boreal forest.
Methods: All sampling was conducted at the long-term common tree gardens located in the Kevo Subarctic Field Research Institute. Soils and litter (stems, roots, senescing leaves) were collected from 3 different birch progenies from Iceland, Finland, and Siberia that have been reported to contain different SPME content (low, medium, high, respectively). Soils and surface litter from beneath each progeny were also collected. We characterized the SPME content of these plant progenies and used a variety of traditional microbiological techniques (i.e. enzyme assays & functional incubation assays) and advanced molecular techniques (i.e. gene-targeted metagenomics) to address the hypothesis that different levels of SPMEs will result in different microbial community structure and function.
Results: Results indicate that microbial populations associated with trees of different SPME content differ in their functional potential (enzymatic and biodegradative). Results also indicated that microbial communities vary in compositing in accordance with dominant tree species present.
Conclusion: Microbial community structure and functional potential appear to be affected by varying SPME content associated with circumpolar boreal tree species.

P SOIL 29

Diversity and functional analysis in Brazilian biomes

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The biosphere, microorganisms have diverse functions in ecosystems and biogeochemical cycles of carbon, nitrogen, sulfur, phosphorus and various metals, but the function of most of these organisms is still unknown. The microorganisms responsible for the existence of these processes are mainly belonging to Bacteria, Fungi and Archaea domains. Understanding the structure, function, stability and adaptation of microbial populations, is extremely important for basic research, biotechnology (search for new genes and products thereof), agriculture, environment and human health, in addition, access to data from DNA sequences these communities, as well as comparisons between gene content and the

community of organisms found. The objective is to evaluate the microbial diversity of different biomes using metagenomic techniques. The total DNA of different soil biomes (Cerrado, Forest, Canga, Grass, Eucalyptus Forest) was sequenced using next-generation sequencers. The result of sequencing of samples were analyzed checking functional analysis and diversity taxonomic classification. The phylum Proteobacteria was the most abundant for all soil, followed by Actinobacteria for Forest and Cerrado, and Firmicutes for Canga, Grass and Eucalyptus Forest. For Archaea, the greatest diversity was the phylum Euryarchaeota for Eucalyptus Forest, Canga and Grass. Functional analysis of the soils showed higher abundance of microbial pathways in a community from Grass soil, however Eucalyptus Forest and Cerrado showed the lower abundance compared to all biomes. Understanding the microbial community and its structure in different soils is of great importance for the understanding of microbial diversity and also to the understanding of biogeochemical cycles or in the search for genes of biotechnological interest.

P SOIL 30

Maize lines with different Nitrogen use efficiency (NUE) differ the molecular diversity of β-glucosidase encoding genes and glycosidase activity in their rhizosphere

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To understand relationship between plant roots and functional diversity of the rhizosphere soil is still a challenge due to the difficulties in precise sampling of this physically restricted, chemically complex and dynamic microenvironment. Here, we studied the changes in microbial biomass, cellulase, N-acetyl-glucosaminidase, β-glucosidase and β-galactosidase activities and molecular diversity of β-glucosidase-encoding genes, in the rhizosphere of two contrasting maize lines differing in the Nitrogen Use Efficiency (NUE). The high NUE Lo5 maize induced larger changes in microbial biomass and enzyme activities than the low NUE T250 maize line. Significant differences in the diversity of β-glucosidase-encoding genes observed between rhizosphere and bulk soil of both maize lines ($p < 0.05$). The *Actinobacteria* and *Proteobacteria* were the dominating phylogenetic group in all samples but representatives of *Bacteriadetes, Chloroflexi, Deincoccus-thermus, Fermicutes and Cyanobacteria* were also detected. Among the *Proteobacteria*, genes clustered only into *α, β, γ* and *δ-proteoabacteria*, with genes from *α-, β-* and *γ-proteobacteria* being dominant in Lo5 maize line, whereas *δ-proteoabacteria* was the most abundant class in T250 maize line which showed that both maize lines induced differences in rhizosphere β-glucosidase-encoding bacterial community and selecting specific populations. We concluded that plants with higher NUE alter the molecular diversity of bacterial β-glucosidase-encoding genes and induce higher C-hydrolasing enzyme activities in the rhizosphere. These results can be useful for better understanding the influence of the used crop plants on the C dynamics in the agro-ecosystems.

P SOIL 31

A degradation pathway for sulfoquinovose in a typical soil bacterium, *Pseudomonas putida* SQ1, *via* a so-called Entner-Doudoroff type pathway

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Agricultural soils are becoming increasingly limited in plant-available inorganic sulfate and a detailed understanding of the recycling of sulfate from sulfur-organic compounds in soils is important. One example of a highly abundant organo sulfur compound relevant in soil is the plant sulfolipid sulfoquinovosyldiacyl glycerol (SQDG), which is produced by all higher plants, mosses, ferns and algae. The polar headgroup of SQDG is sulfoquinovose (6-deoxy-6-sulfoglucose; SQ), which can be mineralized in two steps by bacterial communities: First, SQ is degraded to a C_3-organosulfonate, sulfolactate, and the sulfolactate is excreted. Second, other bacteria utilize the sulfolactate completely *via* a well-defined degradation pathway, and release the sulfonate-group as sulfate. Thus, the sulfur cycle for SQ is closed within a bacterial community. Our aim is to elucidate the genes and enzymes involved in the degradation of SQ in an isolated, typical soil bacterium, *Pseudomonas putida* SQ1.

With proteomic, transcriptional and bioinformatic analyses, as well as through heterologous expressions and enzyme assays with LC-MS analyses, we identified five inducible enzymes and genes for a conversion of SQ to sulfolactate. We termed this pathway the “Sulfo”-Entner-Doudoroff pathway, as it proceeds in direct analogy to the “normal” Entner-Doudoroff pathway for

glucose-6-phosphate. Intracellular SQ is oxidized to 6-sulfo-gluconolactone by a SQ-dehydrogenase, dehydrated to 6-sulfogluconate by a sulfogluconolactonase, and further dehydrated 2-keto-3-deoxy-6-sulfogluconate (KDSG) by a sulfogluconate-dehydratase. The KDSG is cleaved into pyruvate and sulfolactaldehyde (SLA) by an aldolase. The pyruvate enters the central metabolism and sustains growth of *P. putida* SQ1. In a last step, the SLA is oxidized by a SLA-dehydrogenase to sulfolactate, which is excreted.
With the genetic information on SQ degradation, which is now available, the importance of these genes can be evaluated directly in the soil and rhizosphere microbiomes in future work.

P SOIL 32

Fungal-bacterial interplays at biogeochemical interfaces:Co-occurrence of fungi and bacteria in natural and artificial soils

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Introduction: Most soil microbial ecology studies deal with the enormous diversity and functioning of bacteria, whereas the role of mycelial fungi is investigated only to a lesser extent. Thanks to their high surface to volume ratio mycelia take up nutrients and energy sources efficiently (´fungal pipeline´), and with hyphal lengths of up to one kilometer per gram soil build up highly complex systems also permitting bacterial transport (´fungal highway´) (Harms et al., 2011).
Objectives: We investigated the co-occurrence patterns of fungal and bacterial communities in different model soil habitats to study the potential of an interplay of fungi and bacteria during colonization and foraging of spatially heterogeneous biogeochemical interfaces (BGIs) in water unsaturated zones.
Materials and methods: Four sterile artificial soils were inoculated with a water-extracted microbial inoculant from an agricultural soil (Luvisol), fertilized with sterile manure and matured for two years. The soils contained quartz sand and silt in combination with (i) montmorillonite, (ii) illite, (iii) montmorillonite and charcoal or (iv) illite and ferrihydrite. After maturation the artificial soils as well as fresh Luvisol were amended with maize-potato litter, incubated for up to 63 days and the fungal and bacterial communities analysed with high-throughput sequencing.
Results: Community structure analyses revealed distinct, soil composition-dependent colonization patterns for the two microbial groups. Furthermore, the addition of plant litter strongly affected the community composition of the fungi and bacteria. Microbial community network analyses based on Fisher´s exact test revealed distinct co-occurrence relationships between fungi and bacteria in response to the soil composition (i.e. the abiotic microbial habitat) or the addition of plant litter (i.e. resilience after a disturbance).
Conclusion: Our data suggest that BGIs are drivers for bacteria and fungi both during colonization of new habitats, as well as their responses to environmental changes, maybe due to shared niche preferences and helper-effects (´fungal highways´ effect).

Reference
Harms, H., D. Schlosser and L.Y. Wick, 2011. Untapped potential: Exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Micro, 9(3): 177-192

P SOIL 33

Gene capture by hybridization as a strategy for targeted microbial genome reconstruction

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Question: Microbial communities show the greatest organisms diversity on earth. Culture-independent molecular approaches targeting ssu ribosomal RNA genes have revealed this extraordinary diversity. However, amplicons cannot

establish the link between microbial communities structure and realized metabolic functions, limiting the comprehension of micro-organisms roles. Despite the advent of the current ultra-high throughput sequencing, efficient assembly of sequencing data from metagenomic samples remains difficult and complete genome reconstruction has been principally realized for dominant micro-organisms. Single cell sequencing strategies have also been developed to overcome these limitations and to access less abundant micro-organisms but are not always easily practicable. Based on a solution hybrid selection method combined with next generation sequencing, we developed an innovative gene capture approach to enable the reconstruction of large genomic regions or even complete microbial genomes from complex environments.
Methods: A first gene capture using highly specific short probes targeting 16S rRNA gene was applied on metagenomic DNA to obtain large specific probes (several kbp) spanning the biomarker and the unknown flanking regions of the microbial species of interest, *Roseobacter denitrificans*. Generated long probes were then used to capture very large DNA fragments of *R. denitrificans* within a mix of bacterial gDNA.
Results: Real-time PCR performed on captured DNA revealed a significant enrichment in targeted 16S rRNA gene, surrounding genes and even several Mbp away genes, suggesting the capture of *R. denitrificans* complete chromosome.
Conclusions: Sequencing of captured DNA allows identification of unknown sequences and reveals new genetic associations. This promising approach will facilitate linking identity and associated functions.

P SOIL 34
Unrevealing the ecological secrets of important bacterial taxa from forest soil through isolation, whole genome sequencing and single-cell genomics.
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Coniferous forests represent a large C sink in the northern hemisphere and are thus of global importance. The main objective of the present study was to disclose the metabolic capabilities of abundant and active bacterial strains from a *Picea abies* forest soil in the Bohemian Forest Natural Park, Czech Republic. Isolation, physiological characterization and whole genome sequencing of important bacterial taxa in the soil and litter horizons was performed to identify their roles in ecosystem processes. In addition, Single-Cell genomics was applied in order to obtain genomic information from the viable yet unculturable bacteria. In a previous study in the same soil, the microbial community and their metatranscriptomic profile were obtained. Low nutrient medium (pH 4.5) was used to isolate the maximum number of bacterial strains with high abundance in the acidic soil. After identification, enzymatic activity and C-source arrays were used for physiological characterization of isolates as well as whole-genome sequencing from isolates and single cells. Sequencing results revealed that *Proteobacteria*, *Acidobacteria* and *Actinobacteria* were dominant in both the litter and soil, comprising 85-90% of all sequences from DNA and RNA. In total, 299 bacteria were isolated of which two (*Bradyrhizobium* and *Methylocapsa*) rank among the five most abundant OTUs in the soil. *Proteobacteria*, *Actinobacteria* and *Acidobacteria* were the predominant phyla among the isolated bacteria (65%, 17% and 13%, respectively). The physiological characterization of environmental strains showed that soil bacteria are relatively versatile in their ability to use soil-derived carbon sources. Annotated gene content of selected strains indicated presence of CAZy, ammonification and denitrification genes, suggesting these bacteria play a role in important ecosystem processes such as soil carbon and nitrogen turnover. Moreover, individual bacterial genomes were used to identify the transcripts of studied taxa in the soil metatranscriptome. It showed differences in gene expression among seasons for the most relevant bacterial strains. Finally, non-isolated abundant members of the forest soil bacterial community were obtained through Single-Cell sorting, confirming that it can be a valuable tool for microbial ecologists.

P SOIL 35
Rhizosphere microbial community composition of common beans with different levels of resistance to *Fusarium oxysporum*
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Microbial communities in the rhizosphere make significant contributions to plant health, growth and protection against soil pathogens. Plants can take advantage of their rhizosphere microbiomes to fend off pathogens, avoiding microbial infections. Here, we aimed to identify potential microbial groups and functional traits correlated to the suppression of the soil borne

pathogen *Fusarium oxysporum*. Through shotgun metagenomics we investigated the rhizosphere microbial communities of four common bean cultivars with different levels of resistance to the fungus, ranging from susceptible to resistant. Plants were grown in mesocosms experiments with two contrasting soils, *i.e.* Amazon Dark Earth (ADE) and an agricultural soil (AS). The soils presented clear differences in chemical properties, and ADE hosts higher microbial diversity than AS. Chemical analysis indicated a significant increase of pH, Ca, Fe, sum of bases and base saturation, and decrease of K, Mg, exchangeable Al, and Mn in rhizosphere of both soil types. Quantitative PCR showed an increase of 16S rRNA copy number with the increase resistance to the fungus in ADE soil. The rhizosphere of the four bean cultivars is dominated by the same bacterial phyla *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Chloroflexi*, albeit in different relative abundance between soil types. The community structure of rhizosphere was different from the bulk soil, revealing the selection process in this environment. In ADE soil, the most resistant cultivar presented higher taxonomic diversity when compared to other cultivars; in contrast, the functional diversity was lower. Comparing the resistant to the susceptible cultivars there was an increase of *Nitrospirae*, *Solibacteres*, *Spirochaeta* and *Chryosiogenetes* bacterial classes in the resistant. Also, resistant cultivar presented high number of sequences affiliated to the family *Pseudomonadaceae* and to the genera *Bacillus* and *Solibacter*. Interestingly, the resistant and moderately resistant cultivars, presented high proportion of sequences related to bacteriocin, a narrow spectrum antibiotic, which suggests its role on pathogen suppression. Preliminary analysis showed that the selection of the microbial communities inhabiting the common bean rhizosphere is cultivar and soil type dependent. Further analysis will search for bacterial groups potentially related to the fungal antagonism. FAPESP 2014/03217-3.

P SOIL 36

Microbial Diversity in Brazilians Soils

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The soil is a challenging environment with regard to the quantity and diversity of species and microbial communities present, which in one gram of soil is possible to identify thousands of species. However, the interpretation and the way in which this composition, are poorly studied. The use of microbial communities to ascertain the impact caused by anthropogenic stress in natural habitat is increasing. Temporal variations influence the diversity of niches to support microbial populations. The consequent reduction in microbial diversity in soils includes not only the extinction of species or loss of their functions, but to reduce the ability of ecosystems to with stand periods of stress and undesirable environmental effects. In this work, the mainly objective is investigating the bacterial diversity in soil samples with high concentration of iron and manganese by metagenomic techniques. Five soil sample types were collected: grass (CP), forest (MF), eucalyptus forest (ME), canga (CG) and cerrado (CE). Metagenomic DNA was extracted from the soil samples using PowerLyzer® PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc.), and the V4-V5 region of *16S rRNA* gene was amplified and sequenced using Ion Torrent PGM (Life Technologies). The resulting sequences were processed by *MOTHUR* and the FASTA sequences identified were compared with Ribossomal Database Project - RDP for identification. The classification of this phylas in each environment showed that the three most abundant were Proteobacteria, Actinobacteria and Acidobacteria. In Proteobacteria group, the most abundant classes were Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Deltaproteobacteria. Through this methodology was possible to compare the bacterial population of the areas studied, determine the main genus, evaluate the interference and found the environmental impact of this metals, iron and manganese, for the bacterial population and classified genus that have potential for application of bioremediation.

Keywords: Metagenomic, Diversity, Bacteria, Soil, Sequencing

P SOIL 37

Unravelling the functional bacterial diversity from different soils supplemented with organic fertilizer from a Brazilian Zoo Park

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The "*Fundação Parque Zoológico de São Paulo*" (FPZSP) is located in the city of São Paulo, Brazil. It houses *more than 3,200 animals in* an area of 900,000 m^2 of Atlantic Forest. The FPZSP maintains an independent farm designed for the production of food for their animals. The FPZSP Organic Compound Production Unit uses animal carcasses, and animal and vegetables wastes to produce an organic compound (OC). The OC is used as fertilizer in the Zoo farm. This fertilizer displays

a wide microbiological *diversity, which can impact the biogeochemical cycles of the soil and may* propagate pathogenic microorganism. In this study we compared the bacterial diversity of supplemented soils with the OC against non supplemented soil from a native riparian forest. The metagenomic DNA were collected from three different soil samples: native riparian forest soil (N); vegetable garden soil frequently supplemented with OC (G); and soil for forage grass production, which is rarely fertilized with OC (F), over a rainy (April, 2013) and a dry (September, 2013) seasons. The full-length 16S rRNA was sequenced and analyzed by bioinformatics approaches. The garden soil (G) and forage grass production soil (F) microbiome presents a lower diversity compared to the native riparian soil (N) (*Shannon* Diversity *Index* is 3.9/3.6, 3.6/3.6 and 4.8/4.5, respectively for G, F and N samples, in rainy/dry seasons). In addition, no changes were noted with the number of microorganisms involved in the carbon and nitrogen cycles in soils supplemented with OC (G and F) compared to soil N (25 and 30%). Conversely, the microorganisms involved in the sulfur cycle decreased in rainy (13%) and disappeared in dry season, while the phosphate solubilizing bacteria increased (15%) in both season on G and F samples. The F and G shown a enrichment of bacteria involved in bioremediation processes (7%), whereas the pathogenic bacteria decreased (5%). Noteworthy that none bacteria belong to Acidobacteria Phylum was detected in F and G soil, they are observed only in N samples. This corroborate that the OC can change the bacterial natural populations, impacting the soil microbiome despite the economical and environmental importance of its use.(Supported by FAPESP and CNPq; Acknowledgement to FPZSP)

P SOIL 38

Biodegradative abilities of novel fungus isolated *Aspergillus sp*. SIL2014 from environmental habitats soil contaminated automotive lubricants able to use a lot of hydrocarbons as only carbon and energy sources

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In the present study was verified a potential biodegradative abilities, and the bioremediation potentials of fungus specie isolated. Study sites from the soil contaminated by the factory that manufactured automotive lubricants site in Ribeirão Preto County, São Paulo State (Brazil), at 21°06'42.80"S 47°49'0.34"W. The novel fungus described for *Aspergillus sp*. SIL2014 registered in GeneBank with the number KM111287.1. We evaluate the possibility of biosurfactant production through screening for the look for a hemolysis on blood agar. In this study the capability for using as carbon sources diesel S10, kerosene, thinner solvent and acetone. An inoculum was prepared at a concentration of $1x10^7$ UFC / mL equivalent to 0.006 g dry weight was inoculated in the following treatments in minimal medium using as carbon sources: 40; 50; 60; 70; 75; 80 and 85 % of diesel S10, 5 % of kerosene, 4 % of thinner solvent and 4 % acetone during 21 days at 200 rpm and 30 ºC. Absolute biomass measure, such as fungal dry weight showed respectively for each carbon source: 0.74; 0.75; 0.75; 0.76; 0.82; 0.83; 1.11 g for diesel S10, 0.14g for kerosene, 0.02 g for thinner solvent and 0.32 g for acetone (Fig. 1). Fungal Biomass was inoculated on sheep blood Agar plate and incubated for 5 days at 25º C (Fig.2). The results pointed out that the strain is capable to use diesel S10, kerosene, thinner solvent, and acetone for carbon sources. It is concluded that the fungus can be a potential for the treatment of oily residues of petroleum refineries and it can be used for the bioremediation of soils polluted by such compounds. It is proposed however, the necessity of a higher number of studies regarding to the optimization of the efficiency. Financial Support: FAPESP.

Figure 1. The microscopic image produced biosurfactant by novel *Aspergillus* sp. SIL2014, able to use a lot of hydrocarbons as only carbon and energy sources.

Figure 2. Positive strain caused lyses of the blood cells and exhibited a colorless transparent ring around the colony. Hemolysis can also be show with purified biosurfactant.

Figure 1 **Figure 2**

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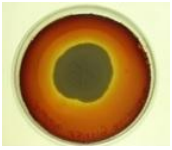

P SOIL 39

Characterisation of *Escherichia coli* B2 strains from waters of Sydney and Gold Coast regions

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Introduction: *Escherichia coli* has a highly clonal population structure, which allows them to be delineated into phylogroups (A, B1, B2, C, D, E, F). Strains of different phylogroups vary in their ecological niche, life history characteristics, and propensity to cause disease. Of all the phylogroups, B2 is commonly isolated from human faeces, and is also the most abundant phylogroup in developed countries. It is clinically significant, as strains belonging to this phylogroup are often the cause of extra-intestinal infections. Although strains belonging to phylogroup B2 are highly diverse, relatively few B2 lineages represent the great majority of B2 strains isolated from humans. Although phylogroup B2 strains are usually a small fraction of the *E. coli* recovered from water samples, their presence may be indicative of human faecal contamination and they may represent human health risk.
Objective: The aim of this study was to determine what fraction of phylogroup B2 strains isolated from water samples belonged to human associated B2 lineages.
Materials and Methods: To this end, over 10,000 isolates of *E. coli* from more than 900 water samples collected in the Sydney and Gold Coast regions were characterised for their phylogroup membership. A total of 825 strains belonging to phylogroup B2 were assigned to one of the human associated B2 lineages using a recently developed allele-specific PCR method for lineage assignment. A subset (291) of the Gold Coast isolates were sequenced type using the CH typing scheme (*fimH*, *fumC*)
Result: Overall, the allele specific PCR method identified 40% of the isolates as potentially belonging to one of the human associated lineages. However, confirmation of subgroup I isolates (putative St131) and subgroup IX isolates (putative ST95) using ST specific PCR based methods revealed that less than 5% of subgroup I and IX isolates actually represented human associated lineages.
Conclusion: The results of this study demonstrate that human associated phylogroup B2 strains represent a small fraction of the *E. coli* strains present in Australian waters.

P SOIL 40

The diversity and abundance of phytase genes (BPP) in the maize rhizosphere

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The ecology of microbial communities associated with phosphorus (P) mineralization in soils is obscure. Here, we assessed the abundance and diversity of bacteria harboring genes from the class *β*-propeller phytase (BPP) genes in the rhizosphere of traditional and transgenic maize cultivated in two Brazilian soils. We found a soil-dependent effect for higher abundance of phytase genes in rhizosphere, and an absence of changes due to plant genotype. Phylogenetic analysis indicated the genera *Pseudomonas, Caulobacter, Idiomarina* and *Maricaulis*, together with *uncultured bacteria*, as the dominant bacteria hosting this gene. The results obtained validated a methodology to target bacteria related to P cycle, indicate the responsiveness of those to the rhizosphere or to the plant genotype, and name the major groups associated with this function in soils, constituting an important step through the deeper knowledge on P-cycling microbes in soils.

P SOIL 41

Edaphic and plant associated factors drive diazotroph diversity and function in Australian soils

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Soil microorganisms are increasingly being recognised for their contribution to agricultural productivity. Non-symbiotic (NS) nitrogen fixation by diazotrophic bacteria is a valuable source of biological N inputs and is highly desirable for the economic and environmental sustainability of nutrient limited agricultural systems in Australia. We measured the effect of edaphic (soil type and environment) and plant associated (plant and variety type - perennial grasses, wheat and barley varieties) factors on the diversity and abundance of *nif*H gene harbouring bacteria in surface soils (0-10 cm) and rhizosphere microbiomes

from cropping regions of southern, western and eastern Australia. Samples were analysed for composition of diazotrophs (*nif*H-TRFLP or amplicon sequencing), abundance (*nif*H qPCR), and the amount of NS-$^{15}N_2$ fixation. A total of 225,000 *nif*H sequences were clustered at 5% amino acid dissimilarity and linking to a curated database gave 40197 OTUs representing 208 unique database matches. The majority of *nif*H sequences (>54%) were related to Proteobacteria; with α- and b-proteobacteria being the dominant members (28-63%). Members belonging to Verrucomicrobiae, Bacillales and Clostrida accounted for up to 5% each. Diversity of N_2-fixing bacteria varied in soils from different agro-ecological regions e.g. diversity was highly correlated to soil related parameters (%clay, pH, organic C) and climatic factors (MAT, MAP). Plant type (grass vs. crop plants) and variety type also influenced the composition and abundance in the rhizosphere microbiome. Different grass species promoted the abundance of specific members of the *nif*H community, suggesting plant-based selection from the soil microbial community. Diversity of diazotrophic bacteria was significantly higher in the rhizosphere than in the roots. Estimates of NS-$^{15}N_2$ fixation ranged from2 fixation regionally and within fields.

P SOIL 42

Metatranscriptomic sequencing reveals differential expression response of paddy soil microorganisms to salt stress

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Introduction: The accumulation of salts, particularly sodium salts, is one of the consequences of desiccation and the main physiological threat to soil ecosystems. However, little is known about how soil microbial communities respond to salt stress at the transcriptome level.
Objective: To understand how paddy soil microbial communities respond and adapt to salt stress.
Materials and methods: Paddy soil slurries amended with rice straw were used as a model system to investigate the microbial community response to salt stress. Triplicate slurries were incubated anaerobically for 6 hours (short response) or 48 hours (late response) with 0, 300, and 600 mM NaCl. Illumina RNA-seq of total RNA and enriched mRNA was used for metratranscriptomic analysis.
Results: *Clostridiaceae* were the most abundant bacterial group at the family level. Salt stress had only a minor effect on their relative contribution to the community 16S rRNA pool. However, clostridial mRNA showed the greatest changes in relative abundance from 12.2±2.0% (0 mM NaCl) up to 23.9±4.1% (600 mM NaCl) in the stress treatments. In late response, clostridial transcripts involved in ethanolamine utilization, bacterial cell division, protein metabolism and RNA metabolism were particularly overrepresented in high salt conditions. *Methanosarcinaceae* contributed most to the community 16S rRNA pool among the archaeal metatranscriptome. The relative abundance of their mRNA increased from 25.4±3.8% (0 mM NaCl) to 37.2±0.1% (600 mM NaCl). This increase in relative abundance corresponded well to an overrepresentation of archaeal transcripts involved in protein metabolism and methanogenesis. Among typical stress response categories, *Clostridiaceae* showed a strong transcriptional response related to “oxidative stress” and “osmotic stress”, while transcripts of *Methanosarcinaceae* were overrepresented in “heat shock”. *Methanocellaceae* were the most abundant group among the hydrogenotrophic methanogens. However, the relative abundance of their 16S rRNA and mRNA strongly declined in response to increasing salt stress.
Conclusions: *Clostridiaceae* and *Methanosarcinaceae* were most dominant and showed the most competitive response. By contrast, *Methanocellaceae* were not able to compete under salt stress.

P SOIL 43

Diversity of bacteria associated with natural vegetation of long-term PCB-contaminated soil

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Rhizosphere microflora benefits from the compounds that plants release into soil through roots. Some of the compounds exuded are structurally analogous to some pollutants and can induce enzymes for the pollutants degradation. In our study, we isolated metagenomes from ten different plant species growing naturally in long-term PCB-contaminated soil and their rhizospheres. Our objectives were (i) to investigate bacterial diversity in the rhizosphere and endosphere; and (ii) to identify plants stimulating bacteria potentially capable of PCB degradation by quantification of degradative genes in the rhizosphere samples. As a control, we used bulk soil from the same locality. Bacterial diversity was analyzed after metagenomic DNA was isolated from samples, and amplicons of prokaryotic 16S rRNA genes were prepared and pyrosequenced. In case of

endophyte amplicon preparation, peptide-nucleic acids inhibiting amplification of mitochondrial and plastid copies were used. The obtained sequences were processed with the mothur software package and with the R-project using vegan package. Data analysis revealed that bulk soil is significantly different from all rhizosphere samples. Data including diversity of endophytic bacteria will be also presented. Using qPCR, we were able to quantify the relative abundance of biphenyl dioxygenase genes (*bphA*) in the samples. The highest 16S rRNA to *bphA* gene ratio was observed in the *Betula pendula* rhizosphere indicating more abundant populations of PCB-degraders. This work brings new insight into the natural microbial populations associated with plants naturally growing in contaminated soils.

Acknowledgements
Funding is acknowledged of the Czech Science Foundation project no. 13-28283S, Ministry of Education, Youth and Sports project no. LH14004 and specific university research MSMT No. 20/2015.

P SOIL 44

Study of bacterial communities in soils of the Sierra Nevada National Park and rhizosphere of a wild thyme species along a thermoclimatic gradient

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Introduction: Given the importance of soil microorganisms in biogeochemical processes and ecosystem functioning, it is important to understand the influence of environmental factors such as climate on the spatial and temporal structure of microbial communities in the soil and particularly in the rhizosphere. The mountain range of the Sierra Nevada National Park in the South of Spain, is a Mediterranean biodiversity hotspot due to its abrupt ecological gradients and diversity of ecological niches. The different thermoclimatic zones at different altitudes together with the presence of cosmopolitan plant species of ecological and economic relevance such as the wild thyme species *Thymus zygis* L., form an ideal study model for climate studies of the rhizosphere.
Objectives: To determine the effect of climate on microbial communities of soils of the Sierra Nevada National Park and in rhizosphere of *Thymus zygis*.
Materials & Methods: The study consists of a spatial and temporal analysis of the taxonomic and functional microbial diversity along a thermoclimatic gradient at different altitudes from two regions (Capileira and Puerto de la Ragua) in the Sierra Nevada National Park during two years. To analyze microbial diversity, metagenomic DNA was isolated from soil and rhizosphere samples and analyzed by multiplex pyrosequencing of amplicons of the 16S rRNA gene. Functional biodiversity was inferred using PICRUSt (http://picrust.github.io/picrust/) which in the future will be complemented by GeoChip analysis.
Results: Current results indicate that the most profound changes in the taxonomic and functional diversity of the bacterial communities are exerted by the rhizosphere of *Thymus zygis*. Although initially differences do not appear to correlate with the thermoclimatic gradient, currently we are searching for possible thermoclimatic specific taxa which may be used as bioindicators to detect climate changes. At the same time we are comparing the dynamics of the bacterial communities to determine possible cyclical changes in the community structures.
Conclusion: The initial results confirm a strong influence of the rhizosphere of *Thymus zygis* on the diversity of bacterial communities irrespective of the sample location along a thermoclimatic gradient in the Sierra Nevada National Park.
Research supported by grants 590/2012 from OAPN of MAAMA and BIO2010-17227 from MINECO and FEDER funds.

P SOIL 45

Characterization of a putative novel *Rhodobacter* species isolated from polluted sediment

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Characterization of a putative novel *Rhodobacter* species isolated from polluted sediment

Being the most widespread inorganic contaminants throughout both artificially and naturally polluted sites and due to their ecotoxicological importance, heavy metals are considered to be a group of pollutants of the highest interest. The research on autochtonic heavy metal resistant microbial communities living in contaminated sites has contributed to the knowledge of how bacteria cope with environmental stress and can also serve as a source of new genetic determinants exploitable for the purposes of bioremediation, biomining, etc. In this work, bacteria from a heavily contaminated sediment (containing 1% of

dry weight of both Cd and Zn) were isolated by selective plating using Cd- and Zn-containing medium. The obtained bacteria were taxonomically classified by 16S rRNA gene sequencing and their Cd- and Zn-minimal inhibitory concentrations (MIC) were measured. Among others, *Microbacterium* sp., *Mesorhizobium* sp., *Devosia* sp., *Vasilyeveaea* sp., *Rhodobacter* sp. and *Pseudonocardia* sp. were identified with Cd- and Zn- MIC values up to 2.7 ± 0.2 and 6.4 ± 0.5 mM, respectively.
Based on the 16S rRNA sequence, one isolate belonging to the genus *Rhodobacter* was recognized to represent a putative novel bacterial species. Therefore, the overall 1422bp-long 16S rRNA gene sequence framed by primers 8f and 1492r was amplified, sequenced, and compared with databases. Using SeqMatch tool from the Ribosomal Database Project and CompGen tool from the EzTaxon server, *Rhodobacter blasticus* ATCC 33485 was determined as the closest type strain exhibiting the similarity score of 95.9% and 94.81%, respectively, both significantly lower than the most recently published threshold value for a new species classification (98.65%, Kim et al., 2014, *Int J Syst Evol Microbiol 64, 346-51*). Morphological, biological and biochemical characteristics of this putative new *Rhodobacter* species will be presented.
The support of Czech Science Foundation is acknowledged (project no. 15-02328S).

P SOIL 46
The role of microbial community in degrading leaves in forest environments.
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Introduction: Soil organic compounds derive from plants degradation, rhizodeposition and the litter fall. Although the leaf breakdown has a key role in the Carbon cycle, the topic is poorly studied especially in the alpine region. Fungi and bacteria play a major role in the degradation of the leaf compounds such as cellulose and lignin which is considered the most recalcitrant substrate. Moreover, there is a general lack of knowledge about the microbiological activity involved in the leaves degradation.
Aim: In this context here, we present a comprehensive study to highlight the role of the decomposing community involved in leaves breakdown in three mountain areas in South Tyrol (Italy) of three different tree species: oak, beech and rhododendron. The species have been selected because exhibit unique characteristics in the chemical composition that is supposed to influence the degradation processes.
Methods: The experiment design considers to introduce for two years replicated litter bags of the three species in three distinct areas at 500, 1000 and 2000 m a.s.l.; a transplantation experimental design is also taken into account. Experiments will consider a metagenomics approach to study the diversity and functionality of bacterial and fungal communities, qPCR to quantify the most important metabolic genes, advanced confocal spectroscopy to highlight the topology of the degradation process, and leaf morphological observations with chemical composition to correlate the microbial dynamics to the status of the process.
Results and conclusion: The project is started in early January and the project overall design with the first results until the sampling time of May 2015 will be shown.

P SOIL 48
The presence of PKS Polyketide Synthase (PKS) and Non-ribosomal Peptide Synthase (NRPS) genes at a deep-sea hydrothermal field in the Aegean Sea
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Deep-sea hydrothermal vents are characterized by extremely high concentrations of microorganisms in stark contrast to the surrounding sea bottom. Nevertheless, deep-sea consumers do not rapidly remove the high biomass of prey from these communities maybe due to vent microbes' chemical defenses which still remain largely unknown. Meanwhile, the detection of genes responsible for antimicrobial and cytotoxic activity such as polyketide (PKS) and non-ribosomal peptide synthases (NRPS) of deep-sea vent bacteria has not so far been attempted.
In this study, sediment and chimney samples were collected from the hydrothermally active field of Kolumbo submarine volcano (500 m depth), in the Aegean Sea, during 2010 E/V *Nautilus* cruise (operated by the Ocean Exploration Trust). Samples were plated on selective media and incubated aerobically for 7 days at 25 °C. The isolated mesophylic bacteria were then tested for antimicrobial activity by diffusion method and strains which exhibited strong activity even after repeated transfer to fresh media, were selected for sequencing, phylogenetic analysis and screening for NRPS and PKS genes using

degenerated primers. 230 mesophylic bacteria were isolated, 42 of which showed remarkably reproducible antimicrobial activity and were affiliated to *Bacillus* and *Proteobacteria*. Based on the conserved Adenylation domain of NRPS and Beta-ketosynthase of PKS, 19 non-ribosomal peptide synthases and 6 polyketide synthesis genes, including *trans*-AT PKSs and hybrid PKS-NRPS, were detected in these bacterial isolates. The presence of surfactant and antibiotic biosynthesis-related to PKS and NRPS genes, suggested the potential ecological role of metabolites produced by the vent bacteria.

P SOIL 49

Greek streptomycetes are recruited to fight as biocontrol agents against phytopathogenic fungi

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The application of biocontrol is truly required by the necessity of the emergence of alternative solutions to handling the plant diseases in general. Streptomycetes are a significant tool in the hands of scientists who look for alternative, ecological solutions to fight against plant diseases caused by microorganisms.
The aim of our work was to examine specifically the biocontrol capacity of the phytopathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum* via Greek indigenous strains of streptomycetes.
A total of 606 streptomycetes, isolated from different habitats of Greeceand belonging to the collection of the Microbiology Laboratory of the Faculty of Biology of the Universityof Athens, have been examined in relation to their ability to control the growth of the phytopathogenic fungi *R. solani* and *F. oxysporum f.sp. lycopersici in vitro*. Culture extracts from the selected streptomycetes have been concentrated and fractionated based on both their molecular weight and their polarity. The effectiveness of the selected streptomycetes in controlling both the symptoms of pathogenic behaviour caused by the fungus *R. solani* in bean plants and cases of wilting caused by the fungus *F. oxysporum f.sp. lycopersici* in tomato plants was also examined through *in vivo* experiments.
Under the criterion of their high anti-fungal activity 12 streptomycetes were selected for further examination. It was shown that the bioactive metabolites are polar compounds that inhibit the growth of each of the two phytopathogenic fungi differently even if they are produced from the same *Streptomyces* strain. The results of the *in vivo* biocontrol trials showed that the most effective biocontrol agent against the phytopathogenic fungus *R. solani* DSM843 was *Streptomyces* GRE 25 (*S. rochei*), while against the phytopathogenic fungus *F. oxysporum* DSM62059 was *Streptomyces* GRE 25 (*S. rochei*) and OL 7 (*Streptomyces sp.*). The most efficient way to use the streptomycetes was by covering the plant seeds with their spores prior planting. The strains GRE 25 and OL 7 could be characterized as growth promoters of the examined plants.
Streptomycetes GRE 25 proved to be the most effective and promising biocontrol agent for the protection from both the target fungi.

P SOIL 50

Effect of biofumigant treatment with defatted seed meals on soil microbial communties

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The replacement of chemical pesticides with alternative biocidal compounds after the Directive 2009/128/EC increased a renewed interest in biofumigation. Defatted seed meals (DSMs) derived from brassicaceae plant tissues with high glucosinolate content represent an alternative to control soil-born plant pathogens and pests, such as nematodes, and can be applied in synergy to catch crop green manures. However the potential impacts on soil microorganisms is still largely unknown.
A plot-scale experiment was set up in pots with tomato plants grown in a naturally nematode-infected soil and treated as follows: i) glucosinolate-containing DSM from *B. carinata* (CAR), ii) non-glucosinolate-containing DSM from sunflower (GIR) and iii) metham-sodium fumigant (VAP).

454-pyrosequencing (NGS) of bacterial 16S rRNA and eukaryotic 18S rRNA genes, was performed at the beginning (T0) and after 5 (T1), 30 (T2) and 60 days (T3) to assess the structure of the microbial communities. Moreover, tomato plants were transplanted and checked for the presence of pests and/or pathogens.
NGS on bacterial communities revealed that the 97% of the ~600 detected species are poorly represented (Proteobacteriawas the most abundant phylum (35.5%) with minimum values at T0 (16.9%) and the highest in CAR sample at T1 (57.8%). Other bacterial phyla, such as *Acidobacteria* (13%), *Actinobacteria* (8.8%) *Planctomycetes* (8.9%) and *Gemmatimonadetes* (5.2%) were also present and their distribution was dependent on the treatments. In general CAR showed similar biocidal efficacy as VAP against the nematodes, but with a negligible impact on bacterial community. The biofumigant action provided by CAR as compared to GIR, suggested how the biocidal effect was unlikely due to the organic fraction of the DSM.
These results confirmed the interesting potential of biofumigant DSM amendments as alternatives to chemical fumigants for a more environment-friendly control of some soil-borne diseases.

P SOIL 51
Tapping Bacterial Resources - Accessing Secondary Metabolites of the Uncultivated
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A large fraction of microbes cannot be cultivated with the current laboratory methods. These microbes most likely produce an enormous diversity of so far undescribed compounds. Several ideas and approaches for tapping the vast resources of novel secondary metabolites exist and include the use of previously neglected habitats, the introduction of novel culturing techniques and the functional or bioinformatics exploitation of DNA from uncultivable organisms.
To evaluate the potential of moss associated communities growing on calciferous places, three moss species growing in black pine forests, *Ditrichum flexicaule*, *Tortella tortuosa* (frizzled crisp-moss) and *Rhytidium rugosum* (wrinkle-leaved feather-moss) were collected from a Lower Austrian forest. The DNA of moss microbial communities was isolated directly from moss and moss rhizosphere samples and from slurries made with the moss plants in minimal medium (VL55) with and without addition of antibiotics (Rif, Ery, Chl and Van). DNA from slurries was isolated from pellets after 4 weeks of incubation. Certain DNA samples will be used for construction of metagenomics libraries. Enrichment of mosses-associated bacteria in minimal media and separately micro-compartment with resistance to antibiotics promises not only to prevent re-isolation of already well known metabolites, but also a depletion of the most common bacteria.
In this study, we will use next generation sequencing (NGS) technology to predict potential in mosses-associated bacterial communities for secondary metabolites production. Based on sequence information, we will study diversity and novelty of secondary metabolites biosynthesis gene clusters from original and slurry enrichment samples. Furthermore we are performing high throughput screening (HTS) of metagenomics libraries for detection of clones that produce biologically active compounds.

P SOIL 52
Elucidating the role of *Microbacterium* spp. in the mobilization/immobilization of heavy metals
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The ability of soil-born and plant-associated bacteria to influence the mobility of metals in soils is a not well understood phenomenon. Through the secretion of various metabolites, bacteria can change the bioavailability of metals. This feature is important not only for the biogeochemical cycles and the bioavailability of metals, but also for the improvement of heavy metals (HM) clean-up techniques like phytoextraction. Indeed, one of the limitations in the application of these clean-up technologies is the plant HM uptake rate dependent on the HM availability.

Although *Microbacterium* spp. is a rather rarely studied actinobacterial genus, HM mobilization by HM adapted members of this taxon has been observed by several independent studies. We could show that *Microbacterium* spp. strains and their exudates increase Zn and Cd extractability from a metal contaminated soil leading to increased plant uptake and accumulation. The absence of acidification led us to the hypothesis that the mobilizing compounds could be secondary metabolites with a metal ligand structure. These features make them suitable candidates for the understanding of HM mobilization processes driven by bacteria.
Therefore, ten *Microbacterium* spp. strains isolated from clean and HM contaminated environments were selected and sequenced to identify genes involved in the HM resistance and im/mobilization processes using RAST and antiSMASH. The presence in the sequenced genomes of polyketides synthases and non-ribosomal protein synthetases supports our hypothesis. In fact, these enzymes are able to synthetize diverse metabolites including metal chelators. Genome comparison and a deeper investigation of *Microbacterium* genes involved in HM resistance and mobilization is currently being performed. The isolates will be screened in HM mobilization assays. Mass-spectrometry (MS) is being used to characterize the metabolites and metal adducts are quantified by ICP-MS. Selected strains will be tested in greenhouse experiments for phytoextraction improvement.
Our results will help to understand adaptation mechanisms to HM enriched or nutrient-depleted habitats and the improvement of HM remediation approaches.

P SOIL 53

Effects of pasture restoration on soil bacterial community in the Cerrados of Central Brazil

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Introduction: An intensive recent occupation process has transformed the Cerrado biome, a savanna in Central Brazil, into the most important region for cattle ranching and intensive plantation commodity crops. Because of this, pastures degradation became an important problem, leading to serious environmental consequences in this biome. Restoring those areas may become strategic, both, for the economical importance, and for the biome preservation.
Objectives: The aim of this work was to compare the bacterial soil communities associated with three areas in Cerrado biome: a native cerrado area, a pasture in use and a pasture planted with tree species. The plantations were mixed with native species, prioritized those of multiple uses: medicinal and food that add value to legal reserves and with important ecosystem functions.
Material & Methods: Bacterial community composition was determined by barcoded pyrosequencing of the 16S rRNA gene. We found that the bacterial community was significantly affected by different uses of the three areas.
Results: Differences in pastures areas probably have been caused by the management for the planting of tree species and the entry of plant material. Of the 17 phyla identified in these soils, nine phyla (Verrumicrobia, Proteobacteria, Planctomycetes, Firmicutes, Chloroflexi, Bacteriodetes, AD3, Actinobacteria, and Acidobacteria) were abundant, and eigth phyla (WPS-2, TM7, TM6, SPAM, Armatimonadetes, Chlamydiae, Elusimicrobia, Gemmatimonadetes,) were considered low-abundance. The phyla Acidobacteria and Proteobacteria and the group “unclassified bacteria” were predominant under all areas, and the phylum Acidobacteria was the most abundant group in both seasons. In the native cerrado area, the bacterial community profile showed a diverse pattern of distribution, without a dominant group. Acidobacteria were dominant in pasture in use and pasture planted with tree species, followed by Firmicutes, Planctomycetes and Proteobacteria.
Conclusion: Our data suggests that changes in the bacterial community could be related to land use and by the differences between the chemical characteristics of the soils under different uses.

P SOIL 54

Search for Highly Copper-Tolerant Soil Bacteria by Cultivation in Micro Segmented Flow

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The search for highly heavy metal-tolerant microorganisms is of interest for understanding the response of natural communities on contamination, for remediation and for general evaluation and classification of soils [1, 2]. The principle of stochastic confinement in micro fluids segments and the cultivation of samples in larger sets of well-separated small

cultivation volumes are well suited for such screenings [3]. The ability of this technique to supply highly resolved dose/response functions [4] was used here for the search of highly copper tolerant bacteria in soil samples from ancient mining areas.
For this purpose, cell and spore suspensions from soil samples have been aliqouted in to micro fluid segments of about 0.5 µL volume with stepwise varied concentration of Cu(II) ions. The growth was evaluated after eight days of incubation by micro flow-through photometry and fluorometry. The principle of application of increased copper concentration in micro fluid segments and a screening example are given in the figure. Bacterial growth was observed up to about 9 mM copper. In many cases, different growth intensities and typical response pattern related to photometric and fluorometric signals have been observed in dependence on copper concentration.

Acknowledgement: For cooperation and stimulating discussions we thank K. Martin, M. Roth and E. Kothe (Jena). The financial support of BMBF (PTJ, Bactocat, Kz. 031A161A) is gratefully acknowledged.

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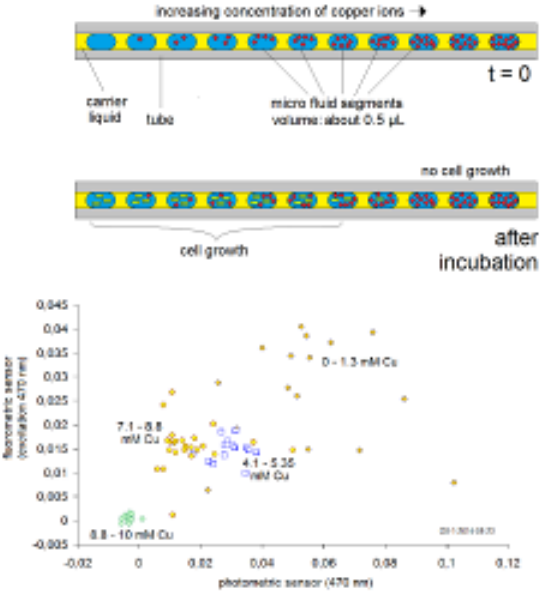
Figure 1



P SOIL 55

Role of soil microbial communities on suppression of plant pathogenic nematodes

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Pratylenchus penetrans is a polyphagous nematode species living in soils and causing major damage to a wide variety of arable crop plants. The role of different agricultural measures (intercropping with marigold plants) and soil treatments (compost, chitin applications, biological soil disinfection) within different agricultural management practices (organic, conventional) on *P. penetrans* decline was investigated in a field site nearby the village of Venray (Vredepeel location) from 2006 on. We hypothesized that changes in microbial fractions in soils caused by differently applied agricultural measures and soil treatments within the two different management practices would impact *P. penetrans* survival in soil and also would affect suppressiveness towards *Meloidogyne* nematode species like *M. chitwoodii*, *M. hapla* and *M. incoginita*. To test this hypothesis we applied suppressive tests with the three *Meloidogyne* species and analysed the soil microbial community structure by next generation sequencing using bacterial (Miseq) and fungal (Pacbio) amplicon sequencing. More than 200 field samples were analysed, obtained from differently treated plots (4 replicates per treatment), over different years (2006 - 2014). Results obtained thus far revealed significant differences in *P. penetrans* densities and suppressiveness against *Meloidogyne* species over different soil treatments and measures within the contexts of the two studied management practices. Also, microbial community structures differed remarkably between different soil treatments, in spite of the fact of large variations that often occurred between replicates within the same treatment. Most obvious observation was that agricultural management practices had different effects on *P. penetrans* densities in soils and on soil suppressiveness towards *Meloidogyne* species. *Pratylenchus penetrans* densities were lowest in organically-managed soils, leading us to conclude that the type of agricultural management practice play an important role in the suppression of nematodes in Vredepeel soil.

P SOIL 56

An effective approach to retrieve culturable *Acinetobacter* spp. from the soil environment

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Question: A basic prerequisite for understanding the role of a given group of bacteria in ecological processes is a comprehensive knowledge of their physiological and metabolic features at the species level. To gather such information, obtaining pure bacterial cultures and their laboratory characterization are inevitable. Bacteria of the ubiquitous genus *Acinetobacter* play an important role in biological processes in soil ecosystems. However, the taxonomic diversity of these bacteria in natural environments is largely unknown. The aim of this study was to design and test an effective approach to recover taxonomically diverse *Acinetobacter* strains from environmental samples.

Methods: A soil sample was homogenized and cultured in a mineral medium supplemented with 0.1% (w/v) of sodium acetate with vigorous aeration at both 25 °C and 37 °C for up to 48 h. The grown-up cultures were streaked onto both non-selective and acetate agar plates and cultured at the respective temperatures. Up to 150 colonies per sample were directly analysed by whole-cell profiling using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics). The obtained spectra were assessed by matching with those of the current Bruker database supplemented with homemade entries representing all known and provisional species of *Acinetobacter*. The spectra were further compared using clustering analysis. The uniqueness of isolates with the spectra distinct at the strain level was verified by RAPD or macrorestriction analysis. Sequence analysis of the *rpoB* gene was performed to define the taxonomic position of strains.

Results: Three samples (A-C) from protected wetland areas in the Czech Republic were analysed. As many as 11 unique *Acinetobacter* strains were isolated from sample A. Of these, four were allocated to three known species while seven represented novel species. Sample B showed an extremely high species diversity: 16 strains were classified as seven known and five new species. Sample C yielded 10 strains belonging to three known and three novel species.

Conclusions: We have developed an effective approach which allows for quick taxonomic screening of hundreds of colonies and enables effective recovery of taxonomically diverse *Acinetobacter* strains from environmental samples.

This work was supported by the Czech Science Foundation, project No. 13-26693S.

P SOIL 57

Seasonal dynamics of soil microbial communities under dominant understory vegetation in spruce swamp forest

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Introduction: Spruce swamp forests (SSF) are unique ecosystems lying between open peatlands and mineral soil forests. From a functional and biodiversity perspective, SSF are exceptional ecosystems which are considered to be important refuges for many boreal forest species sensitive to disturbances. Understory vegetation dominates *Eriophorum vaginatum* and *Vaccinium myrtillus* which create patchy environment in SSF. Presence of Eriophorum or Vaccinium can affect microbes through the quality and quantity of its litter and root exudates. Their input directly affects microbial community, which affects SOM and DOM content.

Objectives: Our two main objectives were (i) to describe the seasonal dynamics of bacterial and archaeal communities in spruce swamp forests under the two dominant understory vegetations (Eriophorum and Vaccinium), (ii) to estimate the effect of dominant vegetation on abundance of methanogens and aerobic methanotrophs, and (iii) to evaluate the effect of dominant vegetation on activity of soil enzymes.

Materials & Methods: To characterize microbial community we sequenced variable region V4 of 16SrDNA using Illumina MiSeq platform. OTU-picking and taxonomic assignment was performed using the QIIME 1.8.0 bioinformatics pipeline. To evaluate abundance of microbial functional guilds, qPCR was employed. Hydrolytic enzymes were analyzed by fluorometric method using 4-methylumbellifferone labeled substrates.

Results: Methanogens (Methanomicrobia, Methanobacteria) dominated archaeal community followed by Thermoplasmata, MBGA and MCG. Relative and absolute abundance of methanogens was significantly higher in Spagnum and Eriophorum than in Vaccinium sites. Eriophorum and Vaccinium sites were enriched by Proteobacteria in comparison to Shagnum sites. On the other hand, Sphagnum control site had higher relative amount of anaerobic bacteria (Firmicutes-Clostridia and Chloroflexi). Hydrolytic enzymes were dominated by phosphatase activity showing probably P limitation which was highest on Sphagnum site.

Conclusions: The co-dominating vascular plant significantly affects the functioning of the ecosystem and soil microbial community composition, including the abundance of microbial functional guilds. All these effects are more pronounced in Vaccinium than in Eriophorum sites.

Figure 1

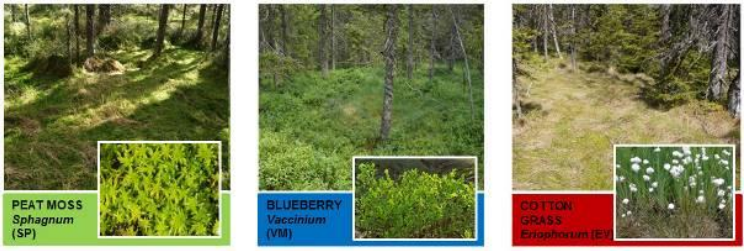
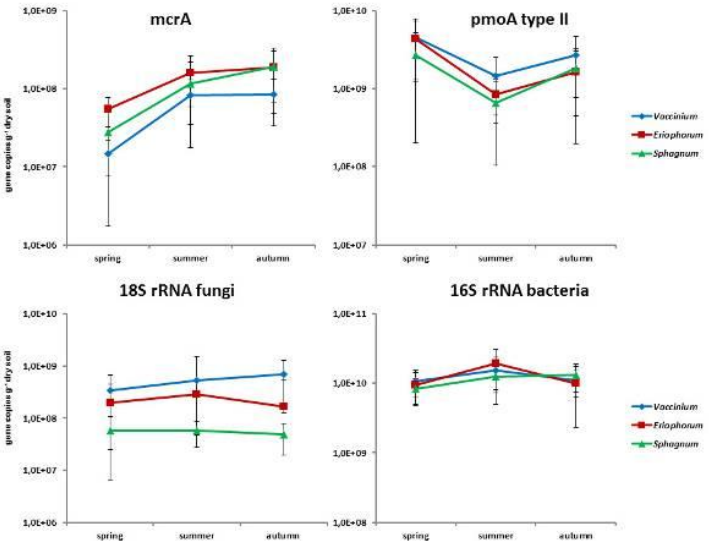


Figure 2



P SOIL 58

Comparison of bacterial soil communities between vineyards and their surrounding semi-natural areas.

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Soil microbial processes play important roles in maintaining soil structure and ecosystem functions where microbiota is influenced by management, plant cover and invertebrates. Comparing the extent of divergence between soil bacteria in vineyards and in semi-natural margins in the same areas was the key objective of this work. Soil samples were collected in 12 vineyards and in their non-cropped surroundings. Farms were located in two neighbouring groups of hills, renowned for their wine quality: the Euganean Hills, with soils of prevalent volcanic origin, and the Berici Hills, with marine sedimentary genesis. Soil bacterial communities were determined based on the 16S rRNA gene analysis through the 454 pyrotag sequencing of the V1-V3 hypervariable region. Soil physical and chemical parameters, fungal communities, and macro- and meso-fauna of the vineyards were also studied, and statistical analyses highlighted interesting correlations between some bacterial taxa and soil characteristics, including the presence of different species of earthworms. Weighted UniFrac distance between soil bacterial communities was calculated, and the related UPGMA tree and PCoA plot showed that the majority of the semi-natural secondary deciduous woodlot soils clustered together, indicating that these, although geographically distant, presented more similar bacterial communities comparing to their respective nearest vineyard soil. Some groups were present with significantly different abundances in vineyard and semi-natural soils: Alphaproteobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Gammaproteobacteria, Gemmatimonadetes, and few other phyla. Within these, some families were decisive in determining differences. Overall, more than 800 bacterial genera and more than 4000 bacterial species were approximated through rarefaction curves. This study provides novel insights into how environment and soil management can affect and

shape soil microbial community composition. This work was carried out within the 'Veneterroir' project, PSR 2007-2013 of the EU funding to Veneto Region.

P SOIL 59

Soil archaeal community changes associated with oil palm fatal yellowing using pyrotags

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Palm oil is considered a potential feedstock for biodiesel production in Brazil due to its high productivity. Fatal Yellowing (FY), of unknown etiological origin, has decimated oil palm plantations in the Amazon region. Little is known about archaeal communities in the Amazon and relative to FY. In this study, soil archaeal communities of oil palm plants with and without FY symptoms were compared using high-throughput sequencing of 16S ribosomal RNA gene (pyrotags). Nine soil samples, grouped according to disease stage (0, 5 and 8), were studied. The Mothur software package was used for quality control of sequences and remaining analysis; the Silva database was used for taxonomic classification. Only two archaeal phyla, Euryarchaeota and Thaumarchaeota were present in all soils studied, regardless of disease stage, with Euryarchaeota being the main phylum in all samples. Euryarchaeota had significant higher representation in soils from plants displaying the most advanced disease stage (Group 8). Soil from Group 8 plants also showed higher archaeal diversity, and the highest number of OTUs. Increasing FY symptoms correlated with a higher representation of genus *Candidatus Nitrosotalea* (Thaumarchaeota) and *Rice Cluster I* (Euryarchaeota) in soil. Although to date there are no reports of pathogenic *Archaea*, this study found an increase in archaeal diversity and an increase in the abundance of specific archaeal in soil from plant with FY. This is the first work to explore a potential role of *Archaea* in plant disease.

P SOIL 60

Soil organic matter mineralization depends on microbial diversity

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Human activities and climatic changes are leading to a rapid and significant reduction of biodiversity, referred to as "the sixth extinction". As a consequence, understanding the effect of diversity on ecosystem functioning and stability is now a central issue in ecological sciences. In this context, we investigated the link between microbial diversity and mineralization of organic matter in soil, which is a major function for soil fertility, environment quality and global changes. To simulate an erosion of soil microbial diversity, microcosms of a soil previously sterilized by gamma irradiation have been inoculated with three different dilutions of a suspension of the same but non sterile soil. By this way, three series of microcosms representing a gradient of soil diversity have been obtained, with D1>D2>D3. After microbial communities have colonized and stabilized, microcosms have been amended with ^{13}C labelled wheat residues. Decomposition of wheat residues and of indigenous organic matter have been assessed during 60 days by measuring $^{13}CO_2$ and $^{12}CO_2$ fluxes, respectively. Results show that intensity of the soil respiration as well as of the priming-effect induced by the addition of plant residues was strongly linked to microbial diversity, with the highest values observed where diversity is greatest.These results illustrate the importance of considering the microbial diversity as a predictive variable of organic carbon storage/release in soils.

P SOIL 61

Non-conventional pretreatments mitigate the inhibitory effect of 5-hydroxymethylfurfural in dark fermentation process.

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Introduction: In dark fermentation process based on mixed cultures, the production of hydrogen (H_2) is carried out by the H_2 producing bacteria (HPB). However, the existing technologies are extremely unreliable and not cost effective. Functional stability and microbial diversity seem to be positively correlated in natural and engineered ecosystems. Thus, understanding

the factors (i.e. pretreatments of the original seed), which shape the assemblage of the HPB community, may help to avoid failures, thereby increasing the efficiency and productivity of the process.
Objectives: This study evaluates the effect of pretreatments on the performance and structure of the enriched microbial community and its potential response to an inhibitor compound (5-hydroxymethylfurfural; HMF).
Materials & methods: Non-pretreated and pretreated (heat shock, aeration and acid) inoculum seed (digestate from an AD plant) was used to inoculate two series of H_2 producing batch tests (C and HMF-reactors). The C-systems (working volume of 100 ml) contained only the seed and glucose (F/M equal to 15) while 1 g/L of HMF was also added into the HMF-reactors. The H_2 production, as well as the level of the intermediate metabolites, was compared and correlated to the composition and diversity of the microbial community assessed by DGGE and Q-PCR.
Results: All systems produced H_2 with a yield ranging between 0.6-0.9 mol H_2/mol glucose. HMF decreased the H_2 production of 19.1±2.9 % in most of the systems. However, in the pre-aerated systems the hydrogen potential was 22% higher than the other systems without significant difference between C and HMF reactors. Extended lag phases were observed in the pre-acidified tests, though the discrepancy of the lag time in the C and HMF tests was smaller than the other bottles. The high performance of the pre-aerated and acidified HMF was due to increased metabolic activity of the main taxa observed in the systems *(Paenibacillus* and *Clostridium* spp.) rather than significant changes in the community composition.
Conclusion: Pre-aeration and acidification of the initial seed can mitigate the negative effect of the HMF. Probably, the initial stressful conditions of the pretreatment modes have activated cell/community response mechanisms able to counteract the toxicity of the HMF.

P SOIL 62

Emissions of Volatile Organic Compounds (VOCs) from soil to the atmosphere depending on agricultural land-uses. Interrelationships between SOM, microbial diversity and VOCs fluxes.

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Soil microorganisms are key players in soil functioning. Indeed these organisms are responsible for the decomposition of SOM and deliver nutrients readily accessible to plants. While the biodegradation of SOM releases CO_2 and CH_4 to the atmosphere, it has been recently shown that microbial SOM mineralization generates VOCs which take part in greenhouse gases and secondary organic aerosols production. The VOC emission rates from soil to atmosphere in agricultural landscapes are still poorly documented compared to CO_2 and CH_4 fluxes.
Our study aims at determining the link between SOM (content and quality), the soil microbial diversity (taxonomic and functional) and the VOC emissions by using a field monitoring approach at the landscape scale. Two long-term environmental research (LTER) were used: the EFELE site from the 'Observation and Experimentation System' (SOERE-Allenvi) and the Zone-Atelier Armorique (ZAA LTER-Europe). At the EFELE site, measurements of VOCs production (quantity and quality) were performed on conventional farming parcels receiving different organic wastes (methanised pig slurry, pig slurry, or bovine manure with nitrogen) and their associated controls (with and without nitrogen). Within the ZAA observatory, a set of contrasted meadows was monitored according to their agricultural management (alternate, permanent and wet meadows).
The VOCs emitted were quantified by using a PTR-MS and identified with a µGC-MS. At the meantime soil was sampled to determine the microbial diversity by metabarcoding on Illumina MiSeq and the molecular composition of SOM by THM-GC-MS. These analyses are still in progress.
So far, five main VOCs produced by the conventional farming plots have been identified: acetone, 2-butanone, dichloromethane, 2-pentanone and decane. Preliminary results indicate also that VOCs spectra and VOCs fluxes differentiated between plots having received or not mineral fertilizers, although this trend has to be confirmed.
In conclusion, modifications of SOM quality and microbial diversity induced by land use (organic amendments, fertilization, grassland management…) seem to influence the VOCs emissions from soil to the atmosphere. VOCs monitoring at the landscape scale will be helpful to implement VOCs fluxes in the Carbone cycle, and to determine suitable land-uses for VOCs mitigation.

P SOIL 63

Diversity analysis and mining of functional genes from soil metagenomes: Case study with ring hydroxylating oxygenases

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Next generation sequencing-based analysis of bacterial phylogenetic markers has become quite reliable and robust. Processing of functional gene amplicon sequences, however, is a lot more challenging than that of 16S rRNA genes and requires cautious approaches. This study aims to investigate different strategies for (i) processing of functional gene amplicon sequences which minimize, or preferably eliminate, errors, and (ii) mining of novel sequences for further functional studies. Our model examples are aromatic ring hydroxylating dioxygenase (ARHD) genes found in soil and stable isotope labeled soil metagenomes.

Combining different processing tools, including a novel method of frameshift detection, we were able to reduce the number of sequences with detected frameshifts from about 10-15% in raw data to none. Different sequences of ARHDs were found in pristine soil metagenomes and metagenomes isolated from aged aromatic pollutants-contaminated soil, pointing out to functional speciation or adaptation of the genes/enzymes to different substrates. Sequences from the pristine soil metagenome were mostly novel, whereas sequences from aged contaminated soil clustered with some previously described ones, although novel clusters were identified as well. Particularly interesting were differences in the substrate specificity-determining regions, which were detected in some sequences in both non-labeled and ^{13}C-labeled metagenomes isolated after stable isotope probing with ^{13}C-biphenyl. Such novel sequences are mined from the respective metagenomes in full length and are cloned in order to determine the substrate range they are able to transform.

Overall, our findings provide novel insight into how functional capacities of a community can be revealed. In addition, using ARHD sequences as a model, we present that novel sequences can be retrieved with different amino acids in the substrate specificity-determining regions.

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P SOIL 64

Rhizosphere microbiome as affected by plant species, Fe nutrition and growth substrates

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Introduction. Plant-associated microorganisms can stimulate plants growth and influence both crops yield and quality by nutrient mobilization and transport. Rhizosphere microbiome appears to be one of the key determinants of plant health and productivity. Plant roots can influence their surrounding microbiology, the so called rhizosphere microbiome, by creating of specific chemical niches through the release of phytochemicals (i.e. root exudates) that depends on several factors, such as plants genotype, soil properties, plant nutritional status, climatic conditions.

Objectives. The aim of the study is to investigate the influence of plant species and the nutritional status on the structure of the rhizosphere microbial communities in two Italian calcareous soils.

Materials and Methods. Two different crops (barley and tomato), with different strategies for Fe acquisition, have been grown in the RHIZOtest system in either complete or Fe-free nutrient solution to induce Fe starvation. Afterwards, plants were cultivated for 6 days on two different calcareous soils. Total DNA was extracted from soil and subjected to pyrosequencing.

Results. NMDS plot well separated microbial communities patterns as indicated by the goodness of fit (0.1) of the stress value for the ordination with two dimensions. The analysis of the bacterial communities confirmed that the two bulk soils showed different structures. The presence of the two plant species, as well as the nutritional status (Fe- deficiency and Fe-sufficiency), could promote a differentiation of the rhizosphere microbiome. *Alphaproteobacteria, Actinobacteria, Chloracidobacteria, Thermoleophilia, Betaproteobacteria, Saprospirae, Gemmatimonadetes, Gammaproteobacteria, Acidobacteria* were the most represented classes in all the samples analyzed even though their relative abundance changed as a function of the soil, plant species and nutritional status.

Conclusions. To our knowledge, this research demonstrates for the first time that different plants species with diverse nutritional status can promote the development of a peculiar rhizosphere microbiome, depending on the growth substrate.

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P SOIL 65

The effect of plant nutritional strategy on the investment into exudation, and the consequences on denitrifying community.

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The rhizosphere is active and dynamic in which newly generated carbon, derived from root exudates, and ancient carbon, in soil organic matter (SOM), are available for microbial growth. Root exudation impact microbial community functioning and especially those involved in nitrogen cycling (Haichar *et al*, 2012). Root exudation is related to plant physiology, which can be measured *via* functional traits. Theses functional traits are used to classify plant species according to their performance. Indeed, fast-growing plant species with higher photosynthetic capacity and rapid rates of N acquisition are called competitive species in contrast to slower-growing conservative species with lower biomass N concentrations but a longer lifespan (Aerts & Chapin, 2000).
The aim of this study was to determine the impact of plant strategy (from conservative to competitive), on denitrifying activity and diversity through root exudation. To do this, we grown in the same soil six grassland poaceaes (2 conservatives, 2 intermediates and 2 conservatives) under $^{13}CO_2$ during one week after 10 weeks of growth on a growth chamber. This labelling allows to estimate the rate of root exudation for each plant and to apply DNA-SIP (stable isotope probing) to identify bacterial community involved in root exudates assimilation in the vicinity of the root system and inhabiting the root-adhering soil and those involved in SOM degradation. In addition, we measured denitrifying activity (Denitrification Enzyme Activity, DEA) to determine interactions between plants and denitrifiers influenced by root exudates.
We have demonstrated that root exudation was linked to plant strategy. In fact, the rate of exudation is most important for competitives and intermediates plants than for conservatives ones, due to a largest root system. In addition, microbial denitrification activity is correlated with exudation rate. The structure and the diversity of bacterial community involved in root exudates assimilation and those involved in SOM degradation analysed by DGGE (denaturing gradient gel electrophoresis) coupled with 16SrDNA sequencing revealed differences in bacterial community using root exudates and those degrading SOM for each plant. To our knowledge, this is the first work demonstrated the impact of plant nutritional strategy on denitrifiers through root exudation.

P SOIL 66

Culturable endophytic bacteria from salt marsh plant *Halimione portulacoides*: functional characterization, phylogenetic diversity and influence of metal contamination

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Halimione portulacoides is one of the most productive and abundant species in salt marshes. This halophyte tolerates and accumulates mercury (Hg) and was proposed as a biomonitor for Hg pollution and phytoremediation purposes. Endophytic bacteria improve plant growth, stress tolerance and are a source of compounds with industrial applications. However, information about endophytic bacteria from *H. portulacoides* is scarce.
We characterized 665 endophytic bacterial isolates of this halophyte from Hg-contaminated sites (B and E) and a non-contaminated site (C), from above (AG) and below ground (BG) tissues, in the estuary *Ria de Aveiro*. PCR-based fingerprinting yielded 467 representative isolates which were identified by 16S rDNA sequencing, and subjected to plant growth promotion (PGP) traits assays.
The collection associated with 4 phyla: Proteobacteria 64%, Actinobacteria 23%, Firmicutes 10% and Bacteroidetes 3%. No site or tissue-level significant differences were found in OTU-based analysis, however there were noteworthy differences in some genera: *Salinicola* spp. isolates (58) were only obtained from site C; *Hoefla* (17), *Labrenzia* (22) and *Microbacterium* (67) were only isolated from BG tissues. Sequence-based UniFrac and principal coordinates analysis of isolates showed that diversity in BG tissues from all sites grouped together, and that the contamination factor was not relevant in the separation of bacterial communities.
Many isolates from the collection tested positive for PGP assays. Remarkably, IAA production was detected in 99% of isolates. Site-level differences in proportion of positive isolates were observed: more proteolytic activity in site C; stronger presence of *nifH* gene in site E; more ACC deaminase activity in site B. Tissue-level differences were also observed: more pectinolytic activity in AG tissues and ACC deaminase activity in BG tissues.

This study explored and exposed structural and functional variations in different tissues of a relevant halophyte in Hg-contaminated and non-contaminated sites.
Acknowledgements: CF grant (SFRH/BD/85423/2012) and PhytoMarsh project (PTDC/AAC-AMB/118873/2010-FCOMP-01-0124-FEDER-019328) were supported by Fundação para a Ciência e Tecnologia (FCT). AA and IH were supported by ESF (EU) and POPH funds (Programa Investigador FCT).

P SOIL 67

Diversity of Novel Alkaline Protease Producing Bacteria from Chilika Lake, Odisha, India.

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Introduction: Chilika lake is the largest brackish water lake in India situated in the east coast of Odisha. The microbial diversity study on Chilika lake was unexplored until recently. Enzymes from marine microorganisms have unique properties and have proven industrial applications. Alkaline proteases are known active ingredients of the commercial detergents and have wide application in leather and tanning industries.
Objectives: Culture-based isolation of alkaline protease producing bacteria and identification & characterization by using polyphasic approach.
Materials & Methods: 30 different sampling sites from the lake were investigated for the isolation of alkaline proteases. Out of 106 isolates, 62 showed protease activity with 2-30% NaCl and pH 5-12.5 by plate assay method. 13 isolates showing highest zone in plate assay within a pH range of 9-11. By using casein as a substrate at pH 10, three bacterial isolates i.e KGS4, KGW1 and PKS7 were characterized by polyphasic taxonomy.
Results: The preliminary phylogenetic analysis based on 16SrRNA sequence and BLAST analysis showed that closest phylogenetic neighbors of KGS4, KGW1 and PKS7 were *Bacillus stratosphericus* 41KF2a(99.5%), *Halobacillus trueperi* SL-5 (99.1%) and *Rheinheimera aquimaris* SW-353(98.58%), respectively. The isolated strains showed alkaline protease activity at pH10 and were morphologically and biochemically distinct from their closest taxonomic neighbors which may be considered as a novel feature. The alkaline protease activities as measured by activity assay of strains KGS4, KGW1and PKS7 were 4.1U/ml, 3.8U/ml, 2.2U/ml respectively, at pH 10. PCR amplified product (1100bp) of *subtilisin e* (EMI14709) gene for alkaline protease was detected in KGS4 .
Conclusion: On the basis of phenotypic, chemotaxonomic properties, phylogenetic analysis, strain KGW1^T KGS4^T and PKS7 should be placed in the genus Halobacillus, Bacillus and Rheinheimera as novel species, for which the *Halobacillus marinus* sp. nov. , *Bacillus kharajalensis* sp. nov. *Rheinheimera pleomorphicus sp. nov*. were proposed. The type strains were KGW1^T (= DSM 29522=KCTC 33609) , KGS4^T (=DSM 29521=KCTC 33610) and PKS7^T (=KCTC 42365)

P SOIL 68

Title: Metagenomic analysis of microbes living in Mars analogue environments

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Assessing the habitability of Mars demands a deep understanding of the occurrence and adaptation of microbial life in extreme terrestrial environments. Collecting anaerobic microorganisms thriving in Mars-analogue sites and the investigation of their response to various Martian stress factors is thus the major aim of the EU- project MASE (Mars analogues for space exploration; more information and all members of the team: http://mase.esf.org/), an international collaboration of scientists with the major aim to cultivate novel anaerobic microorganisms, to perform stress and fossilization tests on them and to apply life detection systems.
Five extreme habitats resembling Martian conditions were chosen: (i) a brine pond, 1.1 km beyond the surface, (ii) cold sulfidic springs in Germany, (iii) a cold, acidic lake in Iceland, (iv) the acidic river Rio Tinto in Spain and (v) a permafrost setting in Iceland.
Besides the cultivation of novel, anaerobic microbes, we will analyze the geological setting of each sampling site. We want to understand how the microbial community is set up in each site and consequently how microorganisms shape these extreme Mars analogue environments - and vice versa.

Sediment samples of every sampling site were taken, part of which were treated with the chemical compound PMA (propidium monoazide) which allows the differentiation between living and dead microorganisms by masking free and accessible DNA in the sample. Extracting DNA of not-PMA and PMA treated samples and metagenomic analysis of each sampling site will give information about which microorganisms would have the capability to thrive under Mars-similar conditions - and which genetic inventory they need to survive.

P SOIL 69

Functional and Structural Characterization of lipolytic enzymes soil metagenomics contaminated with waste lubricant to obtain proteins with high biotechnological potential

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Lipolytic enzymes have show enormous biotechnologycal potential, such as in enzyme mixture for the production of detergents, the processing of leather, the production of cosmetics and other pharmaceuticals, perfumes and biodiesel. Most lipolytic enzymes are derived from microbes, present low toxicity, are easily biodegraded and are notably selective of chemicals. The present work done as an attempt to find genes which codify lipolytic enzyme in fosmid metagenomic library composed of petroleum hydrocarbons degradation microbe consortia. Through the analysis of open reading frames (ORFs) were identified coding regions of four lipolytic enzymes. One of the ORFs (ORF 1) was selected and cloning into vector pET 28a (+), the enzyme was overexpressed *E. coli* C41 (DE3) cells, purified by nickel affinity column chromatography and its identity was confirmed by MALDI-TOF/MS. Stability and lipolytic activity analyses were performed using the spectrometric and titrimetric methods, using p -nitrophenol esters and triacylglycerols as substrates, respectively. To obtain the phase structures were used lipase / esterase with high structural similarity, whose coordinates are deposited at the Protein Data Bank (PDB). Initially, this characterization was based on models of three-dimensional structures built with the help of the Modeller program and using the structural coordinates of proteins 2HM7, 1EVQ and 1QZ3 and later, from the structures solved in the presence of different substrates.The characterization of the interaction of pocket with the substrates is the key point of our project, as from this will be possible to establish strategies for the optimization of protein so we increase their potential biotechnological.

P SOIL 70

Networks and co-occurrence relationships in the lettuce root microbiota

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Garden lettuce is one of the most preferred raw food items worldwide, but occasionally also involved in pathogen outbreaks. The correlative structure of the bacterial community as a network was studied using root microbiota of eight ancient and modern *Lactuca sativa* cultivars, as well as the wild ancestor *Lactuca serriola.* Pyrosequencing of 16S rRNA gene amplicon libraries of the lettuce root microbiota revealed the dominance of Proteobacteria and Bacteriodetes, as well as abundant Chloroflexi and Actinobacteria. Cultivar specificity comprised 12.5% of the species. Diversity indices were not different between lettuce cultivar groups but higher than in *L. serriola***,** which suggests that domestication contributed to bacterial diversification, at least in the lettuce root system. Spearman correlations between operational taxonomic units (OTUs) showed that co-occurrence prevailed over co-exclusion. We applied complementary fluorescence in situ hybridization confocal laser scanning microscopy (FISH-CLSM) analyses to test these patterns, and found evidence for both potential interactions and habitat sharing. Predominant taxa, such as *Pseudomonas*, *Flavobacterium* and *Sphingomonadaceae* rather suggested interactions, although these are not necessarily part of complex co-occurrence modules of more interaction partners. Without the need for complex interactions, single, and even foreign, strains might be able to invade and colonize lettuce plants. This ecological context could also increase lettuce susceptibility to pathogens. Fluorescence in situ hybridization (FISH) was coupled with confocal laser scanning microscopy (CLSM) to study the colonization pattern of the dominant taxa and to confirm the results of pyrosequencing. The combination of co-occurrence analysis and FISH-CLSM allows us to reliably reconstruct and interpret microbial interaction networks.

P SOIL 71

Genome sequencing of Microbacteriaceae spp. with emphasis on heavy metal contaminated environments.

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Microbacteriaceae spp. isolated from heavy metal (HM) accumulating environments (soil, plant tissues) have been shown to play a role in HM mobilization in soil and rhizosphere environments. To obtain insight in the mechanisms involved, we sequenced genomes of selected Microbacteriaceae spp. isolated from non-contaminated and heavy metal contaminated sites.
Isolates from both groups are either plant-associated or soil-borne bacteria: five strains were obtained from the DSMZ collection, whereas the others were isolated from different heavy metal contaminated sites of Europe. Whole-genome sequencing of the extracted microbial DNA was performed using Illumina MiSeq. The raw reads were screened for PhiX contamination and quality-trimmed. Overlapping reads were then merged and long single reads and PE reads assembled with SPAdes. The quality of the genome assemblies was estimated in QUAST and quality control of mapping data performed in Qualimap. Completeness of the genome was assessed using Phylosift and genome annotation was performed with Prokka.
The genome assembly yielded an average coverage of ~100x (n=11). The completeness of the genomes was verified searching for 40 highly conserved, single-copy marker genes of which all 40 were found in each assembly. The phylogenetic analysis of genomes showed no bacterial contamination but the presence of viral sequences instead. Using antiSMASH, it was possible to identify in each genome more than one secondary metabolite gene cluster coding for a broad range of products. Besides terpenoids, lantibiotics and bacteriocines, genes possibly related to HM mobilization process such a non-ribosomal peptide synthetases, polyketide synthases (PKS) and siderophores were also found.
Future comparative genomics studies and the identification of secondary metabolite gene clusters potentially involved in HM mobilization might provide insights into the evolution of specific adaptation to heavy metal enriched habitats and also help us elucidating the mechanisms involved in HM mobilization.

P SOIL 72

Hitherto the analysis of genome fragments from bacteria involved in sulphur cycling in mangrove soils

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In order to unravel the potential metabolic pathways of mangrove sediment microbiome, focused on sulphur cycling, we assessed the fosmidome of microbial communities in an oil-spilled mangrove area in Brazil. A total of 12.360 clones were obtained, from which fosmids were extracted and sequences at Illumina HiSeq2000 and Ion Torrent (total of 2,772,179 of reads on average for each fosmid, with an average sequence size of 112 bp). Assembled contigs (as generated by CLC Bio) were annotated to taxonomic and functional profiles on RAST (Rapid Annotation using Subsystem Technology) complemented by Glimmer3 and Artemis®. The taxonomical analysis identified the bacterial phyla Firmicutes and Proteobacteria as predominant, while Acidobacteria, Actinobacteria, Bacteroidetes, Chlorobi, Euryarchaeota, Ignavibacteriae and Cyanobacteria were present at lower abundances. Annotated contigs were also used to foster our knowledge on the genomic context of S-processing bacteria. It was inferred by analyzing the genome context of *apr* and *dsr* genes, found in two fosmid inserts (23D5 and 40E4, with 31 and 39 kb, respectively). Fosmid 23D5 harbored a core set of essential genes for the dissimilatory reduction of sulfate, but similarities with already described genes was low (25 to 77%). Fosmid 40E4 showed only genes annotated as *dsr*, but not *apr*, also sharing low similarities with sequences in the databases. Comparative analysis low similarities of genes and operon organization with those genes already found in other environments. It might indicate that microorganisms and proteins in mangroves could be resultant from an evolutionary process which is particular to this environment.

P SOIL 73

Plants as drivers of bacterial community structure in aged contaminated soil

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The extent to which plants control the microbial community structure in the rhizosphere had been difficult to elucidate until recently. With the common use of high throughput sequencing technologies, thorough studies of microbial diversity, even in the rhizosphere, have become possible. This study aims to help us understand some key rhizosphere ecology issues, such as to what extent plants shape bacterial community structure, how different community structure develops in the rhizosphere of different plant species, how fertilization affect the structure of the communities, and what are the consequences of the altered community structure in polluted ecosystems. In order to reach our goals, we set up microcosms with aged polychlorinated biphenyl-contaminated soil vegetated by horseradish (*Armoracia rusticana*), black nightshade (*Solanum nigrum*), and tobacco (*Nicotiana tobacum*), and upon a 6-month incubation period we isolated metagenomes and performed 16S rRNA gene amplicon and shotgun pyrosequencing analyses. In addition to plants themselves, the influence of fertilization was evaluated.

Our results indicate that plants, rather than fertilization, shape bacterial community structure and increase phylogenetic diversity. Statistically significant differences were obtained at all taxonomic levels, from phylum to OTU. Functional capacities of the community are also driven by plants rather than fertilization. As far as the bioremediation potential of the communities is concerned, an increased number of reads affiliated with biodegradation of aromatic pollutants was detected in the rhizospheres versus bulk soil.

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P SOIL 74

High taxonomic diversity of culturable Acinetobacter bacteria in the natural soil environment

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Question: The genus Acinetobacter is a phylogenetically broad group of metabolically versatile bacteria which reside in different ecosystems. It currently consists of 37 species with valid names and several provisional genomic species. Although this classification sufficiently covers Acinetobacter spp. isolated from humans, the knowledge of the taxonomic diversity of Acinetobacters confined to non-human environments is very limited. The purpose of our study was to gain insight into the diversity of Acinetobacter spp. cultured from natural soil ecosystems in the Czech Republic.

Methods: Soil samples were subjected to non-enrichment and selective enrichment culture at different temperatures. The former consisted in plating samples on both non-selective and selective (acetate) agar plates while the latter was performed in aerated liquid acetate medium. Acinetobacter colonies were identified based on the genus-specific phenotypic properties and whole-cell profiling by MALDI-TOF MS. Multiple isolates of the same strain were excluded using DNA fingerprinting methods. The final identification/classification at the species level was based on the combination of three validated, Acinetobacter-targeted, taxonomic methods: comparative analysis of the rpoB gene, MALDI-TOF MS and comprehensive physiological and metabolic testing.

Results: We analysed altogether 81 samples collected in well-preserved natural areas scattered over the whole country from 2007 to 2013. Of these, 55 (68%) were positive for Acinetobacter with a total of 167 distinct strains recovered. 113 (68%) of these strains were allocated to 10 known (genomic) species, with the predomination of A. guillouiae (n=34), A. bohemicus (n=30), A. johnsonii (n=23) and A. calcoaceticus (n=16). The remaining 54 (32%) strains were classified into 10 novel taxa or as 17 taxonomically unique strains, which are likely to represent as yet unknown species.

Conclusions: The diversity of environmental Acinetobacter isolates at the species level appears to be unpredictably high and warrants further systematic investigation to provide a taxonomically precise basis for ecological studies.

P SOIL 75

Metatranscriptome-based analysis of hydrolytic microbial communities in an acidic northern peatland

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Sphagnum-dominated peat bogs represent the most extensive type of northern peatlands. Despite the global importance of these ecosystems, the functional capabilities of peat-inhabiting bacteria remain poorly understood. This study was initiated in order to apply metatranscriptomic analysis for identifying microbial populations and enzymes involved in biopolymer degradation in peatlands. The study specifically focused on the degradation of cellulose, xylan, and pectin, which are the main components of *Sphagnum*-derived litter, and chitin, which is derived from exoskeletons of peat-inhabiting arthropods.
Peat sampled from a *Sphagnum* peat bog in northern Russia was amended with 500 mg l^{-1} of the polysaccharides mentioned above and incubated for 6 weeks. Insight into the changes caused by substrate availability was achieved by the analysis of rRNA and mRNA pools via barcoded Illumina sequencing.
Over 81 million RNA reads were generated, of which 36 million reads represented SSU rRNA. Most SSU rRNA transcripts (90%) were of bacterial origin. Substrate-induced changes in community composition were observed for the *Actinobacteria*, *Acidobacteria*, and *Planctomycetes*. Among the *Actinobacteria*, the most pronounced shift occurred in pectin-amended peat due to increase in the *Frankiales* abundance. Changes in the acidobacterial community were most evident for chitin-amended peat and occurred within subdivision 1. The strongest response of the *Planctomycetes* was observed on chitin.
The fraction of mRNA reads was 10 to 17% of the total RNA sequences obtained from peat. Majority of mRNA transcripts (61 to 65%) were of bacterial origin. The functional metatranscriptomes of the substrate-amended peat samples were clearly different from that of a native peat, with all major shifts attributed to biopolymer degradation. The hydrolytic enzymes encoded by the total mRNA transcript pool belonged to 111 families of glycoside hydrolases, 75 families of glycosyl transferases, 15 families of polysaccharide lyases, 14 families of carbohydrate esterases, 62 families of carbohydrate-binding modules, and 11 families of auxiliary activities. This wide repertoire of hydrolytic enzymes corresponds well to the detection of a highly diverse microbial community, which is composed of mostly uncultivated microbial species.

P SOIL 76

Effect of phenanthrene on mobile organic matter and bacterial communities in soil

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The mobile organic matter (MOM) pool in soil comprises microorganisms, organic nutrients and pollutants transported in the soil system. During transport, MOM reacts with solid surfaces forming biogeochemical interfaces (BGIs) which are assumed to be driving sites for ecosystem processes. We hypothesized that the presence of an additional carbon source (model compound phenanthrene) affects MOM and thus BGI properties and functions. We used a two-layer column study in the continuum scale under unsaturated conditions in open-flow mode. Columns (10 x 12 cm) were filled with Luvisol either spiked with phenanthrene in the upper 2 cm (0.2 mg g^{-1}) or left unspiked (control). Columns were irrigated with artificial rain water (1-1.5 pore volume day^{-1}) for 28 days with several flow interruptions of varied duration to allow reactions at BGIs. After the end of the flow period, columns were sliced into several layers for depth-differentiated analysis. The bacterial community composition was studied by denaturing gradient gel electrophoresis (DGGE) and pyrosequencing of 16S rRNA genes amplified from total community DNA which was extracted from effluents and three soil slices. Furthermore, the presence of genes involved in the degradation of phenanthrene and physico-chemical parameters (pH, TOC/DOC, anions, phenanthrene etc.) were determined. A high variation in the mobile bacterial community composition was found by DGGE analysis of effluent samples. Release of MOM was controlled in general by non-equilibrium and only small effects of phenanthrene were observed in the effluent. In the control treatment, minor depth-depending variations were found in the soil bacterial community. In contrast, pronounced shifts in the bacterial community composition and functional gene abundance in the entire soil profile were observed due to phenanthrene spiking which were negatively correlated with the distance to the spiked layer. Major positive responders to phenanthrene were affiliated to the genus *Geothrix* (*Acidobacteria*).
This study revealed strong effects of an additional carbon source (phenanthrene) on the bacterial community and MOM composition in a matured soil. Column experiments conducted with clean artificial soil material are presently analyzed to study the effect of MOM on initial BGI formation.

P SOIL 77

Expression and purification of novel chitinases from metagenomics

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Introduction: Culture-independent metagenomics represents a valuable and promising tool for the exploration and exploitation of the biotechnological potential encrypted in natural microbial communities. Chitinases are of great interest thanks to their potential application in different areas, including (i) integrated pest management strategies, and (ii) production of chitin derivatives.
Objectives: Our work is focused on two genes isolated in the frame of the EU project MetaExplore from two different soil-metagenomic libraries, encoding novel chitinolytic activities [1-3]. Our aim is the production and characterization of the two chitinases (called Chi18H8 and 53D1) using both conventional and alternative heterologous hosts.
Materials and methods: *53D1* and *chi18H8* genes were cloned in *Escherichia coli*. While 53D1 was successfully purified from the soluble cytoplasmic fraction, the latter was recovered from the inclusion bodies. Concurrently, *chi18H8* gene was cloned also in *Streptomyces lividans*, where the protein was secreted into culture broth.
Results: The two enzymes proved to be metallo-containing chitobiosidases, whose activities are influenced by metal ions and detergents. Key features of Chi18H8 include long-term stability in acidic environments, high solvent tolerance, as well as antifungal properties [1, 2], which make this enzyme an interesting candidate as biocontrol agent towards phytopathogen fungi. 53D1 remarkable activity on complex chitinous substrates and its resistance to high salt concentrations suggest its possible application in the treatment and valorization of seafood wastes [3].
Conclusion: The results here presented clearly demonstrate the efficacy of metagenomics for the discovery of novel interesting biocatalysts. The chitinase production in different expression systems will contribute to the development of a metagenome-sourced enzyme expression platform.

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P SOIL 78

Expression and purification of a metagenome-sourced laccase

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Introduction: Laccases are multi-copper oxidases with a broad range of activity towards a variety of phenolic and non-phenolic substances and with several environmental and industrial applications, including bioremediation strategies, the production of second generation biofuels and the decolorization of recalcitrant dyes [1]. Metagenomics, the culture-independent analysis of the genetic complement of entire habitats, represents one of the most promising tools for the identification of novel bacterial laccases.
Objectives: Our work aims at the complete characterization of a putative laccase gene affiliated to the elusive phylum *Acidobacteria* and identified in the frame of the EU project MetaExplore in a metagenomic library made from the bog soil of the Ljubljana marsh.
Materials and methods: The laccase gene was identified using a PCR-based screening approach and affiliated to *Acidobacteria* according to the primary sequence. The predicted ORF was cloned into *Escherichia coli* by using different

expression vectors, alone or in co-expression with a *nosD*-like chaperone gene identified immediately downstream to the laccase gene, thus suggesting a possible role for the *in vivo* production of an active enzyme [2].
Results: Laccase purification was initially hampered by its prevalent accumulation into inclusion bodies in a completely inactive form. Work is on going to optimize purification procedure and to obtain a sufficient amount of the pure enzyme for its biochemical and functional characterization. Preliminary studies suggest that the metagenome-sourced protein is indeed an active laccase, with a prevalent activity on ABTS in acidic environments and at low temperatures.
Conclusion: The obtained results confirm that metagenomics is an efficient tool for the identification of novel bacterial laccases, that, although their high genetic diversity and potential for biotechnological applications, are currently poorly studied if compared to their fungal counterparts [1].

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P SOIL 79
Factors influencing the fate of human pathogens in the plant environment
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Fresh fruits and vegetables contaminated with human pathogens (HP) causing illnesses are increasingly reported. However, so far knowledge is scarce about factors influencing the persistence of HP in the plant environment. In this study, we analysed the influence of the presence of sludge as a nutrient-rich fertiliser and preadaptation of HP on the survival of *Salmonella enterica* serovar Typhimurium LT2. Preadaptation of HP was simulated by cultivation under non-optimal conditions in a new-developed lettuce extract culture medium.
A soil microcosm experiment was carried out including six different treatments: soil amended with sludge or not and each inoculated with *Salmonella*, preadapted *Salmonella* or without inoculum. Soil was sampled regularly, and numbers of *Salmonella* were monitored using culture-dependent and -independent methods. *Salmonella* counts in soil decreased overall from about 10^6 to 10^3 per g dry soil within five weeks. Direct plating showed significantly higher numbers of *Salmonella* in the treatment with preadapted *Salmonella* without sludge compared to the other treatments from 10 days post inoculation (dpi). Significant differences were confirmed by qPCR for 14 and 21 dpi. *Salmonella* were detected in soils up to 175 dpi using PCR-Southern blot hybridisation and enrichment culture with subsequent plating. Furthermore the effect of sludge on the soil microbiome was analysed. Bacterial communities were compared by denaturing gradient gel electrophoresis. 16S fingerprints showed a strong influence of sludge on the soil bacterial community. Inoculated samples showed distinct *Salmonella*-bands on day 0 post inoculation which faded 21 dpi. Soil samples were also analysed with regard to abundance of mobile genetic elements and antibiotic resistance genes at 0, 14 and 119 dpi. Significantly higher abundances of resistance genes *aadA*, *tetA* and *tetW* were found in treatments with sludge at 0 and 14 dpi.
Despite a rapid decline of *Salmonella* in soil our data showed a long-term survival at low abundance. Preadaptation promoted the survival of *Salmonella* in soil while the presence of sludge reduced its survival and had a strong influence on the soil microbial community structure as well as an effect on the abundance of antibiotic resistance genes in soil.

P SOIL 80
Novel insights into the *Eruca sativa* microbiome and the function of *Enterobacteriaceae*
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Arugula (*Eruca sativa* L.) originates from the Mediterranean and is nowadays widely used in traditional Italian cuisine for its peppery, pungent taste and health beneficial effects. As all plants, *E. sativa* is colonized by a vast diversity of microbes at all plant parts. The plant microbiome is contributing to both, plant and human health, although misbalances or pathogen contamination can lead to foodborne outbreaks. Here we studied the structure, abundance and functions of the plant associated microbiota on the phyllosphere, rhizosphere and the corresponding bulk soil in a tripartite approach including next generation sequencing of habitat specific metagenomes and additional enterobacterial 16S rRNA gene amplicons combined with FISH/CLSM visualization. We found highly variable colonization patterns across different microhabitats with a major

divergence for the phyllosphere compared to the soil derived habitats as shown by habitat distances. An overall enrichment of *Gammaproteobacteria* including *Enterobacteriaceae,* and depletion of *Deltaproteobacteria* and *Alphaproteobacteria* on the aerial plant parts and a strong rhizosphere effect was observed. The rhizosphere was depicted as functional hotspot. In contrast, highest alpha diversity indices were found for the bulk soil but with variable functional abundances. A spring embedded network revealed important core taxa of highly enriched *Enterobacteriaceae* including strains related to potential plant and human pathogens. This study provided important insights to understand the nature of bacterial life and ecosystem functioning on *Eruca sativa.* In addition, we were able to shed light on the important role of enterics as plant core member and to unravel sources of pathogen transaction.

P SOIL 81

Screening for ligninlytic enzymes in metagenomic libraries from Caatinga semi arid soil by function and sequence based approaches

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Exclusive to Brazil, the Caatinga Biome is one of the most populated and biologically diverse semi-arid regions in the world. It is located in the Northeast of Brazil and covers 10% of the national territory. This region can be considered an extreme environment due to its high temperatures, low water availability and high ultraviolet radiation. In this sense, it is expected to harbor adapted microorganisms (extremophiles) that can be explored in the search for new genes of biotechnological interest. Soil microorganisms are able to decompose lignin using a set of ligninolytic enzymes (laccases and peroxidases) with potential applications in environmental and industrial biotechnology. However, approximately 99% of soil microbiota cannot be recovered by cultivation methods, thus cultivation-independent methods, such as "metagenomics", are needed for a greater knowledge and exploitation of microbial diversity. The aim of this work was to access the genetic potential of the Caatinga microorganisms by function and sequence-based screening of a soil metagenomic library in the search of ligninolytic enzymes/genes (laccases). Metagenomic DNA from Caatinga soil was used to construct fosmid DNA libraries. A total of 40,000 clones were subjected to high throughput functional screening performed on LB agar plates containing Guaiacol or RBBR dyes, 0,01% L-arabinose, 12.5 µg/mL Chloramphenicol and subsequently incubated at 37°C for 4-7 days. Dye oxidation activity was not detected based on colorimetric assays. On the other hand, using sequence-based approach, degenerate primers (Cu1AF and Cu4R) were employed for bacterial laccase gene PCR amplification using fosmid DNA template. A distinct band (600 pb) was amplified, excised from agarose gel, purified and sequenced. BlastP searches in accurate protein database identified a sequence related to Multicopper Oxidase (MCO) and Putative Laccase. The analysis showed that this sequence is most closely related to *Mesorhizobium* sp. (43% identity). This genus includes species with high geographical dispersion and able to nodulate a wide variety of legumes. Further detailed analysis of the gene arrangement and phylogenetic relationships with other described bacterial laccases are being carried out.

P SOIL 82

Soil and plant associated bacterial communities in two different successional stages of sub-Arctic sand dune ecosystem

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Our research focusses on microbial diversity and their function in the sub-arctic inland primary successional site. The study site is located in an Aeolian sand dune area in subarctic Northern Fennoscandia (68° 29' N). The region belongs to the belt of discontinuous permafrost. In this study, we addressed community composition of soil and endophytic bacteria associated with circumpolar grass, *Deschampsia flexuosa* (wavy hair-grass) growing in two different successional sites. Early successional stage was characterized by the grass *D. flexuosa* growing as monoculture in the blow-out areas. Late successional stage was mountain birch forest vegetation with continuous ground cover vegetation composed of abundant *D. flexuosa* together with *Empetrum nigrum* and the moss *Pleurozium schreberi* under the cover of mountain birch trees. *Deschampsia flexuosa* (leaf and root), rhizosphere and bulk soil samples were collected from four different blow-out areas between 150 and 2250 meters apart. In total, we collected eight biological replicates, two samples per blow out area and

each successional stage. Soil and plant associated bacterial community structure was analyzed using Next generation Ion torrent sequencing of partial 16S rRNA amplicons. Mothur based bioinformatics analysis of 16S rRNA sequences resulted in 179921 quality-filtered sequences. The phylum *Proteobacteria* was the predominant phylum identified and present in all samples. The compositional variation was mainly accounted for by successional stages.

P SOIL 83

Evaluation of the influence of different corn-based cropping systems on the biodiversity of the soil bacterial communities

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The productivity and health of agricultural systems highly depend on the functional processes involving soil microbial communities and an adequate understanding of soil biodiversity plays a key role in ensuring sustainable use of soil. Due to their complexity the mechanisms of how soil bacterial communities are affected by crop rotation are still poorly understood. Therefore, in the present study we evaluated the influence of different corn-based cropping systems on the biodiversity of the soil bacterial communities by analyzing of terminal restriction fragment length polymorphism (T-RFLP) profiles of bacterial 16S rRNA genes. Soil samples were collected in July 2012 from a long-term trial, set up in 1967 at Martonvásár, Hungary. Two plots for four different crop rotation systems were considered: maize monoculture (CR1), 3 years alfalfa and 5 years maize (CR3), 2 years wheat and 2 years maize (CR5), Norfolk type rotation of maize, spring barley, peas and wheat (CR7). The biodiversity of the total bacterial communities in the soil for each trial were analyzed using a Phusion Bacterial Profiling Kit (Thermo Scientific). T-RFLP fingerprints showed high-percentage similarity in each repetition. The highest average numbers of T-RFs were in CR5 trials while the lowest were in Norfolk type 4-crops rotation system. We did not find any significant differences neither in the diversity (H') nor in the evenness (E) of the bacterial communities between maize monoculture (CR1) and the different crop rotation systems (CR5, CR5 and CR7). In order to find clusters of similar groups of samples, a hierarchical clustering analysis of T-RFs was performed. The highest similarity was observed among CR1 and CR5 trials, community composition of 3 years alfalfa and 5 years maize (CR3) clustered close together with them. Surprisingly Norfolk type 4-crops rotation system showed the lowest similarity with the other three trials. Although changes in bacterial communities in response to crop rotations have been documented in several works, we have shown that different crop rotations had no effects on bacterial composition, abundance, and diversity.

This study was supported by grants from the National Research Council (OTKA K101878) and by Research Centre of Excellence (8526-5/2014/TUDPOL).

P SOIL 84

Effect of nitrogen ultilizing effeciencies on rhizospheric proteolytic bacterial communities of two maize inbred lines

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Objective : Effect of root exudates on microbial communities had been investigated by various researchers, still leaving a gap in study of effect on functions related to soil. One such important soil function is proteolysis. This study investigated the interplay of plant nitrogen utilizing efficiency with N mineralization brought about by proteolysis with special focus on bacterial extracellular soil proteases.

Methods: We studied changes in the biochemical activity and microbial community structure in the rhizosphere of the inbred maize (*Zea mais* L.) lines Lo5 and T250 characterized by high and low Nitrogen utilizing effeciencies (NUE) using rhizobox experiments. Plants were regularly regularly monitored for the inorganic N (NH_4^+-N and NO_3^--N) concentration in the rhizosphere by ion selective electrodes (ISE). At the stage of N depletion soil was sampled from rhizosphere and bulk compartments. Cellular biomass was estimated as a measure of ATP and protease activity was assayed as a measure on caseinate hydrolysis. Soil DNA was extracted and was used for molecular studies. Two bacterial genes coding for alkaline proteases (apr) and neutral protease (npr) were selected for studies. Bacterial gene abundance was measured using qPCR. To study diversity of these genes, amplicons were sequenced by illumina high-throughput technology.

Results: Plant L05 with higher NUE, had shown a significant difference in the rhizospheric and bulk cellular biomass, whereas plant T250 with lower NUE showed no significant differences in the biomass content in rhizosphere and bulk soil.

Proteolytic soil potential of plant L05 was found to be higher than that of T250. Furthermore abundance of genes apr and npr and their diversities, as indicated by qPCR and sequencing results respectively were favored by the higher NUE of L05 maize line. Analysis of several million apr and npr amplicons revealed a high diversity of proteases genes in soil and rhizosphere, with many sequences that are still unknown according to current sequences database information.
Conclusions: We found that plant nitrogen metabolism and proteolytic potential of soil are directly or indirectly associated and bacterial proteolytic communities play an important role here. Furthermore, rhizosphere soil exhibits higher abundance of proteolytic bacteria. This study provides useful information to improve the NUE of plants of global agronomic importance even at crop scale.

P SOIL 86
Microbial diversity of different sugarcane vinasse
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The use of vinasse in sugarcane fertirrigation is an established and widely used practice. However, the high cost to transport this material, due to the large volume of water, made them seek economically viable alternatives for use of that product. As a result, to reduce the volume of water the evaporation is used, generating concentrated vinasse. Thus nowadays both normal vinasse (NV) as the concentrated vinasse (CV) are widely used in fertirrigation, particularly to replace the potassium fertilizers. However, another concern is related to the effect of those residues, particularly on the emission of gases responsible for the greenhouse effect, including the N_2O. Some studies characterized NV and CV and their effect in the field, however only few studies focused on microbial communities present in these two forms of vinasse and their relationship with the greenhouse gas emissions. Based on that the present study aimed to characterize strains isolated from NV and CV by PCR approach and screening the genes related to nitrogen cycle. We also investigated the 16S rDNA of microbial communities for possible difference between the different vinasse forms.
Bacterial isolation was done by plating samples of NV and CV on MRS medium. Extracted community DNA was used for amplification of 16S rDNA, *nifH*, *amoA*, *nirK*, *narG*, *norB* and *nosZ*. Ion Torrent sequencing of 16S rDNA was performed to study the bacterial communities present in vinasse.
The six strains obtained from vinasse belong to the Bacilli class. *Lysinibacillus* and *Staphylococcus* strains obtained from vinasse were positive for *nirK* gene, related with nitrogen cycle. Those microorganisms are described as endophytic for several plants, including sugarcane. However, their origin is unclear in present work. 16S rDNA data showed that the microbial diversity is higher in CV with Bacilli class being predominant. In the NV Negativicutes is the most abundant followed by Bacilli. The knowledge of the microbial community present in vinasse will help to better understand the role and importance of those microorganisms in vinasse.
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P SOIL 88
Influence of different phosphate sources on the bacterial microbiome in the rhizosphere and endorhiza of barley (*Hordeum vulgare* L.), investigated by rRNA-deep sequencing
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Introduction: Soil phosphate is often the limiting growing factor of many ecosystems due to scarce mobility, related to the different organic and inorganic forms. Despite its primary role in ecosystem productivity, the effect of different phosphate sources on plant associated bacteria was not investigated yet.
Objectives: To assess the effects of different phosphate sources on both the rhizosphere and the endorhiza bacterial microbiome of barley.
Materials & methods: Barley was grown in greenhouse on nutrient depleted soil amended with each 100 mg P kg^{-1} of either Ca_3PO_4 (CaP), rock phosphate (GAFSA), sodium hexaphytate (NaHex), or without amendment. Total RNA was extracted from both the rhizosphere and the endorhiza of 4 pooled samples per treatment/control. cDNA was obtained by Reverse Transcriptase-PCR of the bacterial 16S rRNAs, and sequenced by IonTorrent. The sequences were analyzed with the QIIME software. Relationships between bacteria and phosphate sources were assessed by correlation network analysis of treatment-specific OTUs.

Results: Phosphate amendment significantly affected the structure of the active microbiome, but only CaP provoked higher diversity indices. Sixty-two OTUs were significantly different between phosphate sources, accounting for 50.6% and 11.3% of the total reads in the rhizosphere and in the endorhiza microbiome, respectively. The co-occurrence correlation network showed that all Nocardioaceae and Acidobacteriaceae OTUs were enriched by CaP, while some Oxalobacteriaceae OTUs were depleted by all phosphate sources and other Oxalobacteriaceae OTUs were enriched by NaHex or GAFSA. Similarly some *Rhodanobacter* OTUs were depleted by all phosphate sources and others slightly enriched by CaP, and *Lysobacter* OTUs were enriched by GAFSA. Negative correlations were also identified, suggesting the occurrence of complementary OTUs.
Conclusions: This is the first study investigating the effect of different phosphate sources on the active fraction of the rhizosphere and endorhiza microbiome. Phosphate source affected more the rhizosphere than the endorhizal microbiome. The most affected taxa were identified and their relationships within and between the two microbial habitats were unraveled.

P SOIL 89
Genomic characterization of a divergent genotype of citrus endophytic *Curtobacterium* strain ER1.6/6
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Considering the diversity of endophytic bacteria and the biotechnological potential of *Curtobacterium* genera, a citrus endophytic *Curtobacterium* strain was characterized by molecular methods. Previous results had shown that all citrus endophytic strains were closely related to *C. flaccumfaciens* pathovars, but they were not able to induce wilt symptoms in bean seedlings. In addition, these analyses indicated that citrus endophytic strains formed clusters that were separate from *C. flaccumfaciens* pathovars, and they are a divergent genotype in *Curtobacterium* genus. Based on their phenotypic and physiological properties as well as their phylogenetic distinctiveness, the citrus endophytic strains could represent a new species or subspecies in *Curtobacterium* genus. The citrus endophytic *Curtobacterium* strain ER1.6/6 was submitted to whole-genome sequencing, which was performed on an Illumina HiSeq 2000 using shotgun approach with 250x coverage. The 10,639,428 paired-end sequences were *de novo* assembled with Geneious assembler (version 7.1.7) and resulted in 22 contigs longer than 1000 bp totalizing a draft genome size of 3,545,085 bp and an N50 of 318,004 bp. The longest contig has 912,315 bp and the overall GC content is nearly to 70.7% in agreement with other Actinobacteria. The presence of 6 hypothetical gene clusters (2 terpenes, 1 Type III-PKS, 1 siderophore, 1 bacteriocin and 1 unknown) was predicted by AntiSmash 2.0 searching this draft genome for secondary metabolites pathways. Once citrus endophytic *Curtobacterium* strains have been previously described as potential agent for biocontrol of *Xylella fastidiosa,* the complete gene prediction and annotation of the genome described above by further pipelines will assist the understanding the mechanisms associated to the ability of this strain ER1.6/6 to control plant pathogens, as well as in comparative genomics studies with phytopathogenical *Curtobacterium* strains.
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P SOIL 90
Microbial dynamics in the soil-rhizosphere interface: from patterns to ecological processes
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An emerging question in Soil Microbial Ecology is whether communities can reach an evolutionary equilibrium after disturbance. Works addressing this issue, often evaluate patterns related to selection, lacking the links with drift, dispersal and speciation processes. This issue acquires importance in the Amazon Biome, due the high rates of deforestation (4,800 km^2 last year), most for agriculture. The aim of this work was to evaluate the microbial dynamics in a long-term chronosequence of forest to no-till conversion (1 to 20-year) and the role of rhizosphere on this dynamics. We performed a soybean mesocosm experiment and characterized bulk soil and rhizosphere microbial metagenomes. Communities became more similar, the extent the chronosequence advanced, in both fractions (Fig1). The α-diversity did not vary across fractions, but increased in both along time, while the β-diversity decreased. *Acidobacteria*, *Nitrospirae*, δ- and γ-*Proteobacteria* showed higher abundances in bulk soil, while *Bacteroidetes*, α- and β-*Proteobacteria* were more abundant in rhizosphere. Microbial trade-offs occurred mainly from 1- to 10-year in bulk soil and gradually increased in rhizosphere from 1- to 20-year. Several functions changed in bulk soil and just a few in rhizosphere, along the chronosequence. Network connections increased in

bulk soil and decreased in rhizosphere, along time (Fig2). Community assembly in bulk soil fitted to neutral model, with low to intermediate dispersal and higher influence of the environment (selection), driven by aluminum concentration and cations saturation, while in rhizosphere, the assembly fitted to niche-based model, with intermediate to high dispersal and lower environmental influence. The role of drift on microbial dynamics was not significant (< 50%). Along time, the increase of taxa was counterbalanced by enhanced shared-taxa into bulk soil and rhizosphere, leading to microbial homogenization. Taxa and functions in bulk soil changed along the chronosequence, serving as reservoir of biodiversity to the plant, mainly driven by selection. Functions in rhizosphere remained stable along the chronosequence, indicating functional resilience on plant-roots, via taxa trade-offs, mainly driven by dispersal. [Funding: CNPq-FAPESP]

Figure 1

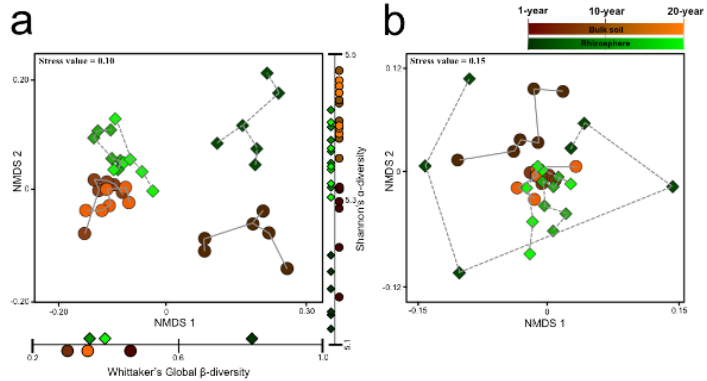
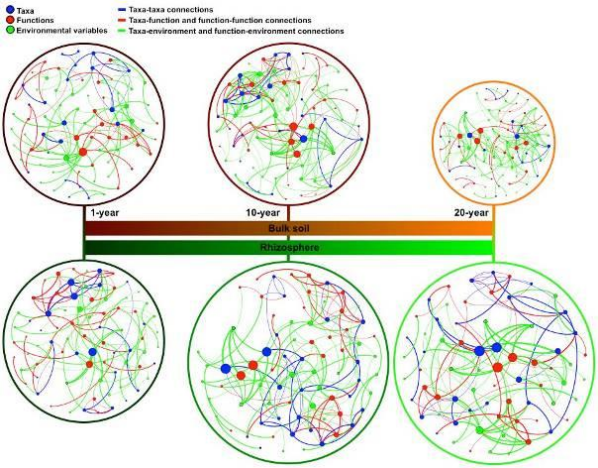


Figure 2



P SOIL 91

Exploring ComQXPA quorum sensing diversity and biocontrol potential of *Bacillus* spp. isolates from tomato rhizoplane

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Bacillus subtilis is a widespread and diverse bacterium which exhibits a remarkable intraspecific diversity of the ComQXPA quorum sensing (QS) system. This manifests in the existence of distinct communication groups (pherotypes) that can efficiently communicate within a group, but not between groups. Similar QS diversity was found also in other bacterial species and its ecological and evolutionary meaning is still being explored. Here we further address the ComQXPA QS diversity among isolates from the tomato rhizoplane, a natural habitat of *B. subtilis*, where these bacteria likely exist in their vegetative form. Since this QS system regulates production of anti-pathogenic and biofilm-inducing substance, surfactin, knowledge on cell-cell communication of this bacterium within rhizoplane is also important from the biocontrol perspective. We confirm the pherotype diversity within *B. subtilis* strains isolated from a rhizoplane of a single plant. We also show that *B. subtilis* rhizoplane isolates show a remarkable diversity of surfactin production and potential plant growth promoting traits. Finally, we discover that effects of surfactin deletion on biofilm formation can be strain-specific and unexpected in the light of current knowledge on its role it this process.

P SOIL 92

Phylogenetic identification of mungbean-nodulating strain MN-S and characterization of its Nod factors

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Question: Biochemical and molecular approaches to identify mungbean nodulating strain MN-S.
Methods: Sequence of 16S rRNA gene and *nodCD1VW* operon were analyzed to identify the mungbean-nodulating strain MN-S. Moreover, Nod factors of various Bradyrhizobial strains were also mass determined as a second line of evidence.
Results: We employed 16S rRNA gene to identity mungbean-nodulating strain MN-S, but in agreement with previous studies, this gene was not found to be a useful polymorphic marker for phylogenetic identification. On the other hand, when we used symbiotic genes from the *nodCD1VW* operon, we learned that two of the four genes generated sufficient polymorphism for the identification of the mungbean-nodulating *Bradyrhizobium* strain MN-S as *B. yuanmingense*. The *nodD1* gene sequence showed 99% similarity to *B. yuanmingense nodD1* and 93% similarity to the same gene in *B. japonicum*. Similarly, *nodC* was found to be 99% similar to the *nodC* sequence of *B. yuanmingense*. However, we were unable to amplify the *nodV/nodW* two-component system genes using a variety of PCR conditions, and thus we conclude that these genes are absent in *B. yuanmingense* MN-S. The *B. yuanmingense* Nod factors were tentatively identified to be in the mass range of 1178 to 1211 Da, which is significantly less than the size of comparable Nod factors from *B. japonicum* USDA 110 and 532C.
Conclusions: On the basis of 16S rRNA, nodC, and nodD1 gene sequence analysis, mungbean nodulating bacterial strain MN-S was identified as *B. yuanmingense*. Likewise, the absence of *nodV/nodW* two-component regulatory system and the production of Nod factors of a lower molecular weight, 1178 to 1211 Da, strengthen the above-mentioned findings and further supplements the literature on *B. yuanmingense*. To our knowledge, this is the first study reporting the absence of a *nodV/nodW* two-component regulatory system and the production of Nod factors of low molecular weights in *B. yuanmingense*.

P SOIL 93

Continuous flooding selects for bacterial populations involved in arsenic cycle in rice rhizosphere

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Arsenic contamination in rice is strictly related to water management. Recent outcomes evidenced that arsenic content and speciation in rice grains cannot be explained only by the metabolism of the plant, but also by the activity of the rhizosphere microbiome. Several microbial species have been reported to perform different arsenic transformations. Furthermore, the activity of iron-reducing bacteria could contribute to the solubilization of arsenic from soil iron oxides.
To test the effect of water management on rhizospheric bacterial populations involved in arsenic cycle, rice plants were grown under different water regimes in a non-contaminated soil (total arsenic 18.4 mg kg^{-1}). The microbial community was characterized by pyrosequencing of 16S rRNA and real time PCR quantification of iron-reducing bacteria 16S rRNA genes. Genes encoding for arsenite oxidase (*aioA*) and for arsenite efflux pump (*ACR3*) were also quantified.
Under flooded conditions arsenate in soil solution increased from 1.40 µg L^{-1} to 190 µg L^{-1}, whereas arsenite increased to 40 µg L^{-1}. Arsenic release was negligible in aerobic rice. Metalloid accumulation in rice grains was 237 µg kg^{-1}, contrary to 4.67 µg kg^{-1} measured in aerobic rice. Rhizospheric microbial populations involved in arsenic speciation were markedly selected under continuous flooding, with relative abundance from 8% in the soil before seeding to 13% before harvesting. In these conditions *ACR3* gene copies increased and were two orders of magnitude higher with respect on *aioA* genes.
Soluble ferrous iron increased from 0.75 mg L^{-1} to 51.1 mg L^{-1} under continuous flooding. In these conditions a concomitant increase of iron-reducing bacteria from 4.6% to 8.7% was observed over time. *Pseudomonas* sp. (4.2%) and *Geobacter* sp. (1.7%) contributed to this increase. Real time PCR confirmed the increase of Geobacteraceae 16S rRNA genes from 10^5 to 10^6 copies (g dry weight)$^{-1}$.
These outcomes indicate that continuous flooding leads to a positive selection in the rhizospheric community of bacterial populations involved in arsenic and iron cycles, promoting the release of arsenic from iron-oxides and the consequent contamination of rice grains.
Acknowledgments: The financial support of the PhD school in Food Systems (University of Milan) and PRIN (2010JBNLJ7-004) was greatly appreciated.

P SOIL 94

Screening for hydrocarbon-producing bacteria from Antartic marine samples

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In the last years, the concern about limited fossil fuels has attracted attention to the development of biofuels from renewable resource. Antarctic continent is the habitat considered source for the recovery of microorganisms with unique metabolic capabilities. Microorganisms can live in this habitat due to the features developed to survive into this permanently cold environment, including changes in composition of membrane fatty acids. However, little is known about the diversity of bacteria associated with marine macroorganisms from cold environment. The goal of the present work was to screen bacteria isolated from marine Antarctic samples for hydrocarbon production, aiming future application in biofuel production.
From several samples collected from Antarctic continent, including sponges, starfish, algae, krill and marine sediments, a total of 196 bacteria was screened for hydrocarbon production. The screening was performed following the method described by Park et al. (2001). The isolates were grown into 3 different media: medium 1 (plate): 2 mg EDTA.2Na; 2.8 mg H_3BO_3; 0.01 g $FeSO_4$; 0.75 mg Na_2MoO_4; 0.24 mg $ZnSO_4$; 0.75 mg $CaCl_2$; 2.1 mg $MnSO_4$; 0.04 mg $Cu(NO_3)_2$; 0.2 mg $MgSO_4$; 0.025 mg thiamine; 0.025 mg biotin; 0.05 mg nicotin; 0.025 mg PABA; 1,64 g acetic acid; 1,92 propionic acid; 1,84 g glycerol; 3.60 g glucose; 1.32 g ammonium sulfate; 0.2 g yeast extract and 24 g Aqua Marine (pH 7,0). After growth, the isolates were transferred to medium 2 (plate): (identical to medium 1, added of 5.40 g succinic acid and 30 g NaCl in place of glycerol and glucose, and Aqua Marine, respectively. After growth, the isolates were transferred to medium 3 (liquid): (identical to medium 2, added of 2.70 g succinic acid and 1.87 g malic acid in place of succinic acid). The isolates that developed a lipid layer on the surface of the medium were considered as positive. All media were incubated without shaking at 5ºC.

From 196 bacteria screened, 18 isolates were selected as putative hydrocarbon producers. The selected isolates will be submitted to analysis of hydrocarbon production by gas chromatography-mass spectometry (GC-MS). These results encourage the development of sustainable biofuels from renewable resources recovered in cold ecosystems.
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P SOIL 95

Characterization of Plant Growth Promoting Bacteria activities

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Microbiome has a key role in many biotic mechanisms which make soil a complex and dynamic ecosystem. In our study we focus the attention on an heterogeneous group of soil microorganisms, named Plant Growth Promoting Bacteria (PGPB), which influence plant development improving nutrient uptake, producing plant hormones and protecting plant against pests.
Our aim is the isolation of PGPB from barley and tomato rhizosphere and the characterization of some activities, such as siderophores and indole 3-acetic acid (IAA) production and phosphates solubilization, to select the best microorganisms to be utilized as biostimulants.
Plants were grown in a RhizoTest System in hydroponical solution, either in Fe sufficiency or Fe deficiency, and then in soil.Microorganisms were desorbed from soil by sonication and isolated by standard dilution plating technique. Two hundred colonies for each sample were screened on CAS agar to evaluate their ability to produce siderophores, which were further purified and characterized by chromatographic and spectrophotometric analyses. Eighty isolates, positive to CAS Agar, were selected and assayed by colorimetric method using Salkowski reagent, to investigate their ability to produce IAA that was further quantified by HPLC. Their capability to solubilize phosphates was evaluated on Pikovskaya agar; quantitative estimation was performed in liquid medium by spectrophotometric method of ascorbic acid.
The eighty isolates were also identified by molecular analysis. A region of the 16S rRNA gene was amplified and sequenced; sequences were aligned to reference sequences available on NCBI by BLASTn. Molecular evolutionary and phylogenetic analyses were performed using Seaview 4 and phylogenetic trees were constructed according to the maximum likelihood method.
Phylogenetic characterization showed no significant evolutionary differences among microbial populations inhabiting the rhizosphere of different plant species, as well as under different iron nutrition conditions. Of the eighty isolates, forty-seven, belonging to genus of *Pseudomonas*, *Azotobacter*, *Rhizobium* and *Enterobacter*, were able to produce IAA and to solubilize phosphates simultaneously; apparently, both plants rhizosphere and iron nutrition status did not affect significantly their activities.

P SOIL 96

Ecology of aerobic and anaerobic ammonia oxidizers communities driven by different soil management in a temperate paddy field

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Most rice agro-ecosystems are characterized by regular flooding, causing alternating redox conditions, strongly affecting nitrogen (N) cycling and fertilizer use efficiency. This may be even more complex as related to crop residue practices. Aerobic and anaerobic ammonia oxidation (ANAMMOX) are two important pathways responsible for part of N losses. However, very little is known on how paddy management regulates their dynamics and functional importance. Therefore, it is crucial to comprehend the microbial regulation of these processes as a function of different crop residue and water management practices.
The research was carried out in four plots within an experimental rice platform (NW Italy) having different straw and water management practices as follows: F-NS: straw removed, flooded; F-S-SPR: straw incorporated in spring, flooded; F-S-AUT: straw incorporated in autumn, flooded; DRY-S-SPR: straw incorporated in spring, dry seeding and 1 month delayed flooding. Soils were sampled during the 2014 rice growing season (May to June) in correspondence to two N fertilizer applications under dry conditions. Chemical and molecular analyses were adopted to study the microbial communities dynamics in terms of abundance and structure.

The abundance of nitrifiers was not inhibited by water management and an evident competition over time between ammonia-oxidizing archaea (AOA) and bacteria (AOB) was observed. The clear temporal distribution of these two groups indicate that AOA predominated over the AOB during field flooding after first cover fertilization, especially in F-NS and DRY-S-SPR. Field flooding after the last fertilization had a positive effect on AOB which showed a higher abundance in all treatments. Anoxic conditions resulted in the coexistence of AOB and ANAMMOX bacteria, indicating that nitrifying bacteria may be the major source of nitrite for the latter. A clear alteration in the communities structure was evidenced, influenced by changes in soil redox conditions and correlated to straw addition. The results suggested that paddy management, especially straw incorporation, affected the nitrification process, driving the creation of different but connected ecological niches. This study is a step forward in understanding the overall microbial dynamics in mitigating the N loss from rice fields.

P SOIL 97

Bacterial assemblages associated with unique root morphology of a desert plant

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Plant-associated bacteria in arid lands have an essential role in supporting plant growth under drought stress. To evaluate the contribution of the root microbiome to the growth of desert-adapted plants, the bacterial diversity associated with *Panicum* sp. growing in the sand dunes of the Tunisian Sahara was studied. This xeric plant presents unique root morphology with the central root covered by a cylindrical sand grain rhizosheath. This is composed of mucilage produced by the root metaorganism (the assemblage of root tissues and the associated microorganisms), root hairs and sand/soil particles. These components are presumably involved in increasing moisture absorption and limiting desiccation. Electron microscopy techniques suggested the presence of bacteria on rhizosheath sand grains and root hairs, the distribution of which was confirmed by fluorescence *in-situ* hybridization. Culture-independent and -dependent analysis of bacteria associated with the rhizosheath and root tissues also showed a difference in the spatial distribution of bacterial genera according to the specific root portion, suggesting that the plant determines selective pressures that shape the observed morphology. Metaphylogenomic data showed that root tissues were dominated by *Gammaproteobacteria* mainly represented by the *Pseudomonas* genus. *Actinobacteria* belonging to *Micrococcaceae* and *Streptomycetaceae* families were enriched in the rhizosheath. The isolates associated with the *Panicum* rhizosheath and root system, including oligotrophic and halotolerant strains, presented multiple plant growth promotion (PGP) activities *in vitro*. The most versatile PGP strains capable of exopolysaccharide production were tested in microcosm experiments demonstrating their capability to enhance sand wettability.
The complex nature of the microbiome associated with the *Panicum* sp. root system suggests that bacteria are adapted to the environmental conditions of the plant rhizosheath, that appears to attract and select unique root-bacterial communities adapted to drought conditions and contributing to water stress resistance.

P SOIL 98

Bacteria, mineral soil and pioneer plants: a complex interaction in successional stages on Alps

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Introduction: Retreating glaciers offers a unique chance to study the role of bacterial communities in barren rock colonization processes and soil formation.
Objective: Define the role and composition change of bacterial communities since deglaciation.
Methods: we examined four transects in the moraine of the Weisskugel Glacier (2500 m a.s.l) in the Central Italian Alps at 5, 50, 150 and more than 150 years after deglaciation. Bacterial community structure and diversity analysis have been done by ARISA, pyrosequencing and MiSeq sequencing (> 20 million reads) of 16S rRNA gene and of *nifH* gene. Bioinformatic tools were used to assess the bacterial functionality (Picrust).
Results: The estimated number of species in soils after 5 years and 50 years from deglaciation was significantly high (448±58 and 558±32 respectively). The genera prevailing in the soil after 5 years included *Ferrithrix*, *Micrococcus*, *Serratia*, and *Finegoldia*, while after 50 years *Stella*, *Kaistia*, *Luteolibacter*, and *Arenimonas*. Some of these genera were also

predominant in varnish of surrounding rocks surfaces. We compared the most common floristic associations in soils of 150 and more than 150 years after deglaciation (*Cetrario Loiseleurenion*, *Nardion strictae*, *Festucetum halleri*) by total rhizobacterial DNA and RNA analysis in two different seasons. The total rhizobacterial communities were significantly different from the correspondent active communities both in structure and functionality. Within the total bacterial communities, each plot differed significantly according to the sampling season and soil age, whereas within the active bacterial communities plots clustered mainly according to the season. A marked shift in active Proteobacteria, Acidobacteria, Planctomycetes highlighted the difference between the vegetation plots, growing seasons, and soil age.
Functional analysis of all the transects showed specific traits according to soil age with *Bradyrhizobium* as main bacterial genus in N-fixation.
Conclusion: a complex dynamical succession has been highlighted, where bacteria and floristic communities interact together. The ultimate consequences of the interaction strongly depends to soil age, fertility and plant cover.

P SOIL 99
A pan genome analysis of fluorescent Pseudomonads in sugarcane soil and rhizosphere
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Pseudomonas is a large genus known for its ubiquity in the environment, including soil, water, and plant surfaces, and is one of the most effective in colonizing the rhizosphere. Certain strains act by suppressing plant diseases in agricultural and natural environments. The ecological and metabolic characteristic of this genus is huge, not surprisingly extending to the genomic sequence level. Despite its importance in soil, so far few genomes have been sequenced. Here we aim at describing the pan genome (the sum of the shared and strain unique genes across all the compared genomes) of fluorescent Pseudomonas, so we can understand better the ecological relations that these organisms establish in the soil. Differences in the unique genes of close related bacteria could be a partial answer of local adaptation to particular or niches (i.e. bulk soil versus rhizosphere). To do so, we obtained, so far, 34 isolates of *Pseudomonas* in Pseudomonas Agar Base medium (21 from bulk soil and 13 from rhizosphere). The 16SrDNA sequencing revealed the presence of three groups; two in both environments (*P. fluorescens*/*P. koreensis* and *P. plecoglossicida*/*P. monteilii*), while the group affiliated to *P. putida* was exclusively found in the rhizosphere. BOX-PCR analysis indicated a deeper variation on genome contents of these isolates, clustering 7 genotypes (some exclusive in soils or rhizosphere composing 23 BOX profiles). We will further sequence the complete genome of such isolates using Illumina HiSeq platform, following assembly and annotation. With this approach we hope to determine the stable regions in the genomes as well as those more prone to alteration determined by niche occupancy. This study will greatly increase the knowledge of the complex association between genetic background and ecology of these species, allowing innovative inferences about the microbial role in sugarcane development. We will also gain insights on how to use these communities to benefit sugarcane production, through a sustainable plant production system.

P SOIL 100
Archaeal and bacterial metabolically active cells depending on soil depth and land use
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The method of *in situ* hybridization using fluorescent labeled 16S rRNA-targeted oligonucleotide probes (FISH - fluorescence *in situ* hybridization) combines identification and quantification of groups of microorganisms at different phylogenetic levels, from domain to species. The FISH method enables to study the soil microbial community *in situ*, avoiding plating on nutrient media, and allows to identify and quantify living, metabolically active cells of *Bacteria* and *Archaea*. The hybridization can be visualized under the fluorescent microscope and counted. The application of FISH was demonstrated by the abundance of metabolically active cells of Archaea and Bacteria depending on soil properties, depth and land use. The research was carried out at field and natural ecosystems of European part of Russia. Samples were collected within the soil profiles (3-6 horizons) of Chernozem and Kastanozem with distinct land use. Quantification of metabolically active cells in virgin and arable Chernozem revealed that the abundance of Archaea in topsoil of virgin Chernozem was doubled as compared with arable soil, but it leveled off in the deeper horizons. Plowing of Chernozem decreased an amount of archaeal and bacterial active cells simultaneously, however, *Bacteria* were more resistant to agrogenic impact than *Archaea*. In Kastanozem, a

significant change in the abundance of metabolically active cells due to plowing was detected only within 40 cm soil layer, and this effect disappeared in lower horizons. The *Bacteria*:*Archaea* ratio decreased with depth from 7.10 to 3.71 for virgin soil and from 5.74 to 4.11 for arable soil. A relationship between soil organic carbon and the amount of soil metabolically active *Bacteria* and *Archaea* cells revealed that distribution of both *Bacteria* and *Archaea* throughout the soil profile was governed mostly by the organic matter content. Thus, the soil organic matter content was a main factor of declining the *Bacteria*:*Archaea* ratio with depth. As a result, *Archaea* out-compete *Bacteria* under conditions of reduced energy supply.

P SOIL 101

Sugarcane harvest management alters soil bacterial communities

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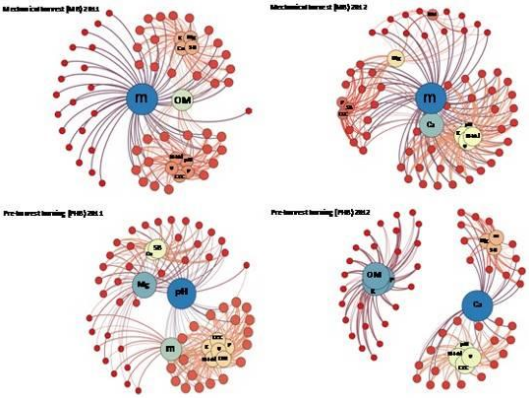
We used networks analysis based on soil chemical factors and high-throughput sequencing of bacterial community from total soil DNA (Illumina MiSeq platform) in order to investigate the impact of two different harvest management - mechanical harvest (MH) and pre-harvest burning (PHB) - in sugarcane fields on soil bacterial community. Based on analyses of soil chemical factors, soils from sugarcane fields under MH and PHB formed significantly distinct groups as indicated by *R* value ($R = 0.95$, $p < 0.05$). Phosphorus content and base saturation revealed significant difference ($p < 0.05$) between sugarcane-cultivated soils under MH and PHB management. Across 12 soil metagenomic datasets, soils from sugarcane fields under MH and PHB revealed differential relative abundance for *Proteobacteria* (36% MH and 38% PHB), *Actinobacteria* (35% MH and 35% PHB) and *Firmicutes* (11% MH and 7% PHB). Taken together into networks, soil chemical factors and relative abundance of taxonomic groups of soil *Bacteria* showed disparate association patterns for sugarcane-cultivated soils from fields under MH and PHB (Figure 1). The number of nodes and edges differed among the networks accounting 65 nodes and 234 edges in the network MH 2011, 65 nodes and 270 edges in the network PHB 2011, 65 nodes and 285 edges in the network MH 2012, and 65 nodes and 208 edges in the network PHB 2012. Aluminium saturation (m) and organic matter (OM) content were the chemical soil factors most related to taxonomic bacterial groups in sugarcane-cultivated soils under MH, with 'm' revealing mostly positive correlations with taxonomic bacterial groups in these soils. In turn, the soil pH, OM and macro- and micronutrients were the chemical soil factors most related to taxonomic bacterial groups in sugarcane-cultivated soils under PHB, with the latter establishing positive and negative correlations with taxonomic bacterial groups in these soils. *Alphaproteobacteria,* an abundant bacterial class in sugarcane-cultivated soils under MH, revealed positive correlation with OM and negative correlation with pH in soils from sugarcane fields under PHB management. These results showed a clear effect of sugarcane harvest management on the soil bacterial communities and their association with soil chemical factors in bulk soil from sugarcane fields.

Figure 1. Bacteria-soil association networks of two different harvest management - mechanical harvest and pre-harvest burning - in sugarcane fields. Soil samples were collected from sugarcane fields in two different years (2011 and 2012).

Figure 1



P SOIL 102

Temporal study of phytoremediation revealed a secondary succession of bacteria and novel extradiol dioxygenase genes in hydrocarbon pollution

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In Finland over 24000 polluted sites are known and in whole Europe approximately 1.2 million. The pace of characterization and remediation of sites is too slow leaving the cleanup for future generations. Detoxification of contamination can be provided by microbial communities. Bacteria have the propensity for rapid evolution of enzymes and corresponding degradation pathways under selective pressure. We studied the temporal and spatial patterns of bacterial communities in hydrocarbon contaminated soils and rhizospheres in ecosystems ranging from controlled microcosms, aged polluted site to long term field study. Massive 454 pyrosequencing of structural and catabolic marker genes coupled with advanced geostatistical and phylogenetic analysis allowed interpretation of the ecological response of bacteria to pollution. Geostatistical analysis brought out niche partitioning as the major mechanism regulating spatial distribution of bacterial communities in the aged polluted ecosystem. In temporal studies we observed a secondary succession of bacterial communities at both structural and functional levels. An integrated analysis of 16S rRNA, alkane hydroxylase and extradiol dioxygenase marker genes revealed co-occurrence patterns of bacterial groups in early and late phases of pollution. Detailed functional gene data displayed unknown clusters of extradiol dioxygenases showing limited knowledge of key catabolic enzymes. The methodological approach gave novel insights to phytoremediation and tools for monitoring of the remediation process.

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P SOIL 103

Multiple semi-continuous chemical detection by bacterial bioreporters in a microfluidics chemostat

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Whole-cell bioreporters have been successfully applied for the detection of different target chemicals, but the development of robust and reliable bioreporter assays remains challenging. In particular, storage of bioreporter cells in such a way that they maintain and display immediate activation potential is a longstanding issue.
Here we propose a strategy that consists in culturing reporter cells in a PDMS-based microfluidic chemostat. Cells are continuously grown in a nL-reactor with integrated valves operated by pressurized channels in a second PDMS layer. The overflow of cells leads to a continuous supply of actively growing cells, which can be exploited for bioreporter assays.
To demonstrate the feasibility of a chemostat-driven biosensor-unit, we used an *Escherichia coli* EGFP-reporter strain for the detection of arsenic, a harmful chemical contaminating water supplies across the world. We show how reporter cells continuously grown in the miniaturized reactor remain in physiologically active state and how part of them can be released from the reactor and transferred to a measurement cage where they are trapped and exposed to an arsenic aqueous sample. Cells stored on chip for one week remain clearly inducible and, as the trapping is reversible, multiple measurements can be performed on the same device.

P SOIL 104

Characterization of test of actinomyctes from different culture systems from Ouargla(Algeria) .

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Several soil samples, taken from Hassi Benabdellah region (Ouargla), sampled from different culture systems were analyzed. An enumeration of microflora, isolation and purification of some actinomycetes were realized. The salt tolerance, the antimicrobial activity and the degradation of sawdust were also studied. The obtained result revealed a moderate rate of microflora. The microscopic study of 20 selected and purified strains, revealed that all of them belonging to *Streptomyces.* The antimicrobial (antibacterial an antifungal) activity secreted by 7 isolates of *Streptomyces* was investigated. The strain *Streptomyces* sp. OG15 inhibits the growth of *Aspergillus carbonarius* and *Fusarium oxysporum* f. sp. *albedinis*. Also an antibacterial activity against *Escherichia coli* was observed. However, we noticed the absence of anti-yesat activity (against *Candida albicans*). The results revealed that *Streptomyces* isolates torelate up to 5% NaCl but any strain torelates concentration of 12,5%. The tolerance of *Streptomyces* isolates is diffent for concentrations of 7,5 and 10%. Degradation of sawdust, after one month incubation, showed some colonies of non-mycelial bacteria and two fungul colonies. However, no mycelial bacteria weres observed. Our results indicated that the early stages of degradation of the studied matrix (sawdust) was made by non-mycelial bacteria and fungi. To conclude this work it should be noted that according to our study, the different cropping systems did not have an effect on the quantitative distribution of microorganisms, actinomycetes represent an average rate in the soils of Hassi Benabdellah. Anlysiz this of soil samples showed e low in organic matter with a mildly alkaline pH and a low average of salinity. The results let us say there is a correlation between the physico-chemical analyzes and the results of microbiological analyzes.

P SOIL 105

The dynamics of physical phenomenon and
chemicals in the high plateaus: the case of the province of Algeria Tissemsilt (soil, water, erosion, pollution)

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The High trays are a case of ecosystem most affected by Algeria in the degradation and pollution of its soil resources. It is a sandy clay mountainous area covering more than 700,000 ha, characterized by a significant wind activity and therefore a strong erosive power. Additionally to this erosive effect, the case of the province of Tissemsilt is subjected to a high urban concentration and various economic activities, agricultural, industrial and port that threaten its resources as its basic ecological balance. This work is a contribution to the diagnosis of the state of degradation of our study area subject to various agricultural and industrial constraints and which are subject natural resources soil and water. One important result of this approach is that the degradation and exists in several forms at the same time it remains undervalued because it has not benefited enough attention from scientists or even socio-economic operators. She opened, however, a process of investigation of paramount importance on ecological and environmental impacts of rapid development that knows the region in the medium and long term.

P SOIL 106

Microbial communities from a transect of non-contaminated to highly hydrocarbon-contaminated soils in King George Island, Maritime Antarctic

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Natural environments are negatively affected worldwide by petroleum spills. Antarctica represents one of the last remaining pristine zones on Earth, thus understanding the ecological consequence of petroleum spills in this environment is essential.

Therefore, the aim of this study was to understand microbial oil impact in Antarctic sites. Soil samples from a transect of non-contaminated to highly hydrocarbon-contaminated soils were collected around the Brazilian Antarctic Station, located at King George Island. Deep sequencing of community 16S rRNA genes and shotgun metagenomic sequencing were used to characterize the indigenous microbial communities' petroleum degradation potential. The 16S rRNA gene sequencing revealed a successive change in the microbial community according to the contaminant gradient. The richness and diversity of the Bacteria and Archaea (based on Chao1 and Shannon-H index) decreased as the concentration of oil contamination increased. The most prominent effects of hydrocarbon contamination levels were observed in Crenarchaeota, Acidobacteria and Gemmatimonadetes phyla, which the relative abundance over the microbial community increase in low and non-contaminated soils. By contrast, the genus *Cytophaga* was detected only in highly hydrocarbon-contaminated soils (11.9%), and the abundance of *Methyloversatilis* progressively increased from 5.43% to 27.2% as the concentration of hydrocarbons increased. The genera *Polaromonas* and *Williamsia* were dominant (up to 13.54% and 12.52%, respectively) in both medium and low hydrocarbon-contaminated soils. Analysis of metagenomic data showed that sequences related to house-keeping genes of *Polaromonas* were mostly found in metagenomic data obtained from medium and low hydrocarbon-contaminated soils, reaching 18.2% of the metagenomic reads.

P SOIL 107

Microbial taxonomic structure of different soil types of Russia

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Introduction: Estimates of microbial species per gram of soil vary between 2000 and 8.3 million. The enormous diversity and ecological diversification makes soil microbiome the essential natural object for the analysis of soil ecological state. However, this approach requires an analysis of microbiomes of different soil types in different conditions.

Objectives

The analysis of microbial communities of main soil types of Russia to reveal the main determinants of the soil microbiomes patterns.

Materials and methods: Soil collection included samples of the top 20-cm layer (A horizon) of podzolic, gray forest soil, brown soil, chernozems, brown soil and saline soils. Samples were collected in conditions of different phytocenosis. DNA isolation was perfomed according to the original methodics. Pyrosequencing was carried out by using 454 Technology by JS Juniour ("Roche", Germany). The bioinformatical and taxonomic analysis was perfomed by using Qiime 1.8.0 software.

Results: It was revealed that the physical and chemical factors such as pH, temperature and moisture content, are the main factors influencing prokaryotes biodiversity. Soils of the southern regions with low hydrothermal quotient (HTQ) were dominated by *Actinobacteria* while in soils of the northern regions (high HTQ) *Proteobacteria* prevailed. Soils with low pH were characterized by *Acidobacteria* proportion increasing.

Analysis of soil microbiomes under different plant communities showed that plant community type plays subordinate yet significant role in a specific prokaryotic pattern formation: the differences were observed at the level of lower taxa rank - orders, families and genera. Grassland microbiomes diversity were generally greater than that of soil microbiomes in tree and shrub phytocenosis.

Conclusion: According to the results obtained the correlation between abiotic factors (pH, HTQ), vegetation type and taxonomic structure of microbial communities was revealed. However, the confirmation of these laws requires the development and implementation of integrated approaches considering the microbial community as a functional unity, which, on the one hand, is entirely dependent on environmental conditions, and on the other - is an active factor of its formation.

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Oral presentations

O NOVI 1

Biodegradation of naphthenic acids by *Rhodoccocus* spp.

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Naphthenic acids (NAs) are an important group of organic pollutants comprised predominantly of saturated aliphatic and alicyclic carboxylic acids. These compounds are toxic, recalcitrant, and persistent in hydrocarbon deposits (petroleum, oil sands bitumen, and crude oils) (e.g. oil deposits of the Athabasca region in Alberta, Canada). The study of the diversity of bacteria able to degrade NAs along with the mechanisms by which NAs are biodegraded provides the basis for the development of bioremediation strategies to decrease both the abundance and toxicity of the NAs in the environment.
The aim of the present work was to study the biodegradation of NAs using bacteria belonging to *Rhodococcus* genus. These bacteria have peculiar features including metabolic versatility and environmental stress resistance. In particular, the utilization of NAs by *Rhodococcus* sp. BCP1 and *Rhodococcus opacus* R7 as sole carbon and energy sources was studied and the effects of these compounds on the physiology of the cells were assessed. By means of reverse transcriptase real time PCR (RT-qPCR), the expression of genes induced by model NAs was studied and a random mutagenesis library was created to define the genes involved in the NAs biodegradation process in *Rhodococcus*.
As a result, *Rhodococcus* spp. were able to utilize NAs such as cyclohexane carboxylic acid (CHCA) and cyclopentane carboxylic acid (CPCA) as the sole carbon and energy sources, at concentrations up to 1000 mg/L. The growth on NAs caused effects on the morphology of the cells and on the cell membrane composition. A gene cluster coding for b-oxidation enzymes was induced during the growth on NAs and the level of expression was dependent on the concentration of substrates added to the growth medium. On the basis of both the transcriptional induction experiments and the mutant library screening a putative NA degradation pathway was proposed.
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O NOVI 2

Co-oxidation of dimethylsulfide to dimethylsulfoxide by marine heterotrophic bacteria using trimethylamine monooxygenase

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Dimethylsulfide (DMS) is a volatile organosulfur compound and plays an important role in climate regulation and the global biogeochemical cycles of sulfur between land and oceans. Over the past decade, research has suggested that in marine surface waters DMS is predominantly transformed into dimethyl sulfoxide (DMSO) through photo oxidation or biological transformation. Although it is clear that microbial oxidation of DMS to DMSO represents a major sink of DMS in marine surface waters, little is known about the biological mechanisms or major contributors to DMSO formation. We have previously demonstrated that trimethylamine (TMA) monooxygenase (Tmm), which is widespread throughout the oceans being present in approximately one in five bacterial cells in the upper oceans, can also oxidise DMS and its affinity for DMS is similar to that of its natural substrate, TMA. The marine model bacterium, *Ruegeria pomeroyi* DSS-3, was used to investigate the potential molecular mechanism governing DMSO production in marine surface waters. A suite of physiological and classical molecular genetic approaches were employed to determine if Tmm-containing bacteria can co-oxidise DMS through Tmm. In this study, it was revealed that Tmm-containing heterotrophic bacteria of the marine *Roseobacter* clade can oxidise DMS to DMSO and that this DMS oxidation is methylated amine-dependent. In addition, it was revealed that the expression of Tmm is regulated post-transcriptionally. Using *Ruegeria pomeroyi* DSS-3 as a model, we show that DMS released from dimethylsulfoniopropionate cleavage was completely oxidised to DMSO in the presence of TMA. Taken together, these observations identify a previously unknown pathway for the biological transformation of DMS to

DMSO in ecologically important marine heterotrophs, and suggests a novel link between the marine carbon, nitrogen and sulfur cycles.

O NOVI 3

Biogeophysics as a Tool for "Smart Sampling" during the Microbial Degradation of Hydrocarbons

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Techniques such as ground-penetrating radar, electrical resistivity and magnetic susceptibility measurements are established geophysical methods to evaluate subsurface environments. We are using magnetic susceptibility measurements to guide us to "hot zones" of microbial activities during the degradation of hydrocarbons in underground oil spills. Studying an aged petroleum plume ~ 10 m beneath the surface that resulted from a pipeline break 35 years ago in Bemidji, Minnesota, US, we found that magnetic susceptibility values are especially high in the zone of the fluctuating water table that moves the free-phase petroleum plume up and down according to seasonal variations. This zone is also the depth of high microbial hydrocarbon degradation activity and the transition between iron-reducing and methanogenic conditions. To evaluate which microbial populations are associated with the zone of elevated magnetic susceptibilities, samples from soil cores were analyzed with denaturing gradient gel electrophoresis, 16S rRNA gene libraries and high-throughput sequencing. The main microbial populations found in the iron-reducing zone included hydrocarbon-degrading *Firmicutes* and iron-reducing *Betaproteobacteria*, whereas in the methanogenic zone syntrophic *Deltaproteobacteria* and methanogenic Archaea predominated.
What is the connection between geophysics and microbial ecology? A magnetic susceptibility probe measures the degree of magnetization of the surrounding sediments. The mineral magnetite, which is iron (II,III) oxide, shows one of highest magnetic susceptibilities. The formation of magnetite can be biologically induced, occurring when high iron(II) concentrations are present together with ferrihydrite. Several bacterial species and some Archaea are able to reduce iron(III) to iron(II) and, under the right pH conditions, may contribute to magnetite formation. Therefore, microorganisms impact geological processes that can be measured with geophysical tools. Because it is an immense problem to comprehensively sample microbial communities along space and time gradients, we suggest that geophysical methods can guide microbiological sampling to zones of microbiological interest, enhancing our knowledge about natural and anthropogenic induced biogeochemical cycles.

O NOVII 1

Characterization of anaerobic BTEX-degrading enrichment cultures from groundwater along a pollutant gradient

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Monoaromatic BTEX compounds are among the most abundant contaminants in hydrocarbon-polluted aquifers (BTEX: benzene, toluene, ethylbenzene, *o*-, *m*- and *p*-xylene). Compared to other hydrocarbons, BTEX are relatively volatile and have a high solubility in water. They lack reactive groups and are hardly degradable by microorganisms, especially under anaerobic conditions, which prevail at contaminated sites. The aim of this study was to investigate nitrate-dependent BTEX degradation along a pollutant gradient within the plume of a leaking coal tar pit of a former gasworks site. Groundwater was sampled from three wells and batch-incubated with additional nitrate and single BTEX compounds (200 µM) under anaerobic conditions. Degradation rates were compared and bacterial communities were characterized by DGGE, 16S rRNA sequencing and analyzed for the transcription of key-enzymes by RT-PCR. BTEX degrading microorganisms were preferentially enriched in groundwater from a high (> 100 µM BTEX) and a moderate contaminated well (10-30 µM BTEX): Ethylbenzene and toluene were degraded within 5 to 10 days, followed by *p*-xylene (25-40 days) and *m*-xylene (60 days). When using groundwater from a low contaminated well (BTEX < 5 µM), no *p*-xylene and *m*-xylene degrading microorganisms were enriched. On all sites, *o*-xylene and benzene were not degraded. The stoichiometric consumption of 5-6 mol nitrate for 1 mol BTEX implicates the involvement of denitrifying microorganisms. Several *Azoarcus* species were identified and isolation attempts resulted in highly enriched cultures dominated by single *Azoarcus* species. Gene transcripts for enzymes known to participate in anaerobic BTEX degradation such as benzylsuccinate synthase and benzoyl-CoA reductase were detected. This study suggests that the presence of BTEX degrading microorganisms depends on the concentration and the degradability of the respective substrate: BTEX, which are degraded fastest (toluene and

ethylbenzene), are utilized within a wide range of the plume. Slowly degradable BTEX (*p*- and *m*-xylene) are only utilized close to the source of the plume where contaminant concentrations are above a certain threshold value. The finding of considerable degradation rates for *p*-xylene is noteworthy as to date no nitrate-reducing pure culture exists.

O NOVII 2

Unusual bacterial community assembly of a simplified consortium reductively dechlorinating 1,2-dichloroethane

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Halogenated compounds are extensively used in industry and agriculture. Their recalcitrance to degradation pose them as primary issues for public health. 1,2-dichloroethane (DCA) is considered among the most important halo-organics due to its diffusion, being an intermediate for the production of PVC. Health issues associated with DCA, put the achievement of successful bioremediation approaches as well as the identification of the microbes able to degrade it and the metabolic pathways involved as primary goals, since the accomplishment of the successful remediation of a polluted site is dependent on the knowledge of the key microbes equipped with the relevant catabolic genes. The aims of this work were the evaluation of the DCA biodegradative potential of the resident microbiome of an aquifer polluted with high levels of DCA, as well as the enrichment of bacteria directly involved in the dehalorespiration process. We present the characterization of the bacterial community originated from the contaminated aquifer and its development in anaerobic microcosms after biostimulation with different e^- donors. Moreover series of subsequent enrichments cultures were established.We have assessed the structural analysis of the bacterial communities involved in the degradation making use of several 16S rRNA-based molecular methods such as clone libraries, DGGE, ILLUMINA as well as flow-citometry. Following the biostimulation treatment the bacterial community underwent a notable change, with the enrichment of representatives of the order *Clostridiales*. On the other hand the enrichment cultures showed a gradually increasing dichlorinating performance, up to 50 ppm of DCA day^{-1} degraded. A gradual simplification of the community was observed resulting in the achievement of an enriched dehalogenating consortium, with two strains potentially involved in the contaminant depletion, a *Geobacter* sp. and a *Pseudomonas* sp. Neither of them was previously associated to DCA reductive dehalogeantion. *Psedomonas* sp. was isolated from the dehalogenating consortium and showed no chlororespiring capability when inoculated in aneaerobic as well as aerobic cultures with DCA, suggesting *Geobacter* sp. as the putative dehalogenating bacterium.

O NOVII 3

Metagenomic and metagenic approaches applied to enhanced anaerobic reductive dechlorination of polychlorinated biphenyls: linking structure and function

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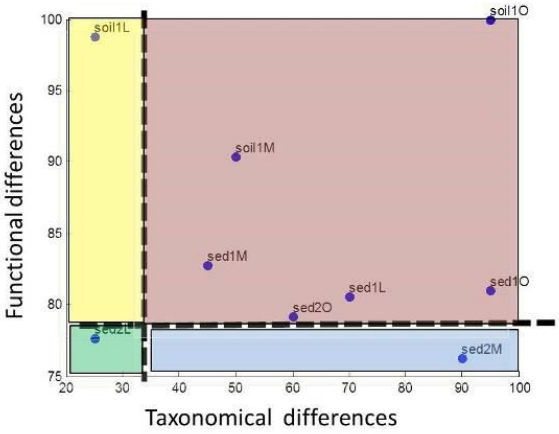
Bacterial adaptation to xenobiotic compounds could occur by the selection of existing biodegraders in the microbial community (taxonomical adaptation) or by the acquisition of novel functions in the bacterial community (functional adaptation). In the first case, the by-products produced by the first biodegraders could be degraded by other bacteria. In the second case, the new degrading function will lead to novel metabolic pathways in one or several members of the microbial community.We use the model of polychlorinated biphenyl (PCBs) degradation to studying microbial adaptation. PCBs can be completely degraded by bacterial communities under sequential anaerobic and aerobic conditions as anaerobic reductive dechlorination is more effective with highly (chloro-) substituted PCBs (leading to the production of less dioxin-like PCBs). Anaerobic conditions were induced in one soil and two sediments polluted with PCBs at different concentrations by the addition of one of the substrates: molasses, lactate or soy bean oil. These substrates are known as potential enhancers of anaerobic dechlorination. To access bacterial diversity and community structure, metagenic approach based on 16S rRNA genes was used. In addition, PCB composition and total mass were measured. Two different metagenomic bioinformatics approaches were applied based on shotgun sequencing: 1) Reads were compared to sequences known as affecting dehalogenation processes. 2) A non *a priori* method was done by comparison with bacterial databases. Correlations were calculated between our metagenic (diversity/structure) and metagenomic (potential functions) results. We were able to

reconstruct partial pathways that may be involved in the dechlorination process. Metagenomic and metagenic together can provide details concerning microbial community involvement in metabolic pathways, and correlate difference between taxonomic and functional adaptation.

Figure 1



Poster presentations

P NOV 1

New Antibiotic Resistance genes from a soil metagenomic library and their unexpected application in bioremediation processes.

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The collection of antibiotic resistance genes of an environment is denominated its resistome and the main reservoir of these genes is the soil, a biodiverse and unappreciated environment in this context. The consolidation of metagenomics field allowed for the identification and description of new genes and functions of cultivable and not yet cultivable microorganisms. Description of the soil resistome and the elucidation of the antibiotic resistance mechanisms in soils will provide more information about the role of resistance genes - and of antibiotics - in nature. As such, the identification of new antibiotic resistance genes from the environment is a first step to access the diversity of these genes in soil, to preview other possible functions of these genes as well as analyze further biotechnological application such as in bioremediation. The screening of metagenomic libraries, constructed with Brazil's Cerrado soil samples, revealed three ORFs similar to dioxygenase enzymes, which are widespread in soil microorganisms, and are responsible for the degradation of aromatic compounds. These compounds are both of natural and external sources, the former ones being extremely recalcitrant in nature, comprising diverse pollutant substances. The final aim was to access the function of theses ORFs in aromatic metabolism as well as their contribution in the antibiotic resistance phenotypes observed in the isolated metagenomic clones. We will show the diversity of the antibiotic resistance genes in the environment as well as their impact in other cellular metabolisms, microbial ecology and biotechnological industry.

P NOV 2

Purification and characterization of a novel carbofuran hydrolase from the carbofuran mineralizing *Novosphingobium sp.* KN65.2

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Question: Carbamate insecticides are broad-spectrum insecticides that comprise a significant proportion of agricultural insecticides used in today's agricultural industry. Carbofuran is one of the most toxic carbamate pesticides and its widespread use has raised health concerns. Most carbofuran degrading bacteria only transform carbofuran into carbofuran phenol without subsequent further mineralization of the phenolic metabolite. Up to now, only a few bacterial isolates, most of them Sphingomonads are reported to mineralize carbofuran beyond carbofuran phenol. Here, we report the identification of a novel carbofuran hydrolase CehA from the carbofuran mineralizing *Novosphingobium sp.* KN65.2, isolated from Vietnamese soil [1].
Methods: The function of the CehA protein was predicted from its gene sequence derived from the draft genome sequence of KN65.2. The protein was purified as active enzyme from KN65.2 crude protein extracts as well as heterogeneously expressed in *E. coli*. The catalytic activity was determined for carbofuran as well as other carbamate pesticides by UPLC.
Results: In contrast to a previously characterized CehA protein from the carbaryl degrading *Rhizobium* strain which can only hydrolyze carbaryl [2], the KN65.2 CehA is capable of degrading both carbofuran and carbaryl as shown by the build-up of the metabolites carbofuran phenol and 1-naphthol. Interestingly, CehA from strain KN65.2 shows only four amino acid substitutions compared to CehA from *Rhizobium* indicating a short evolutionary path between both enzymes. Data on the comparison of the enzyme kinetics and substrate range of both CehA proteins are currently generated and will be presented.
Conclusion: CehA is a novel carbofuran hydrolase that transforms carbofuran to its phenolic metabolite carbofuranphenol in *Novosphingobium sp.* KN65.2 and is likely to be the first enzyme of the carbofuran degradation pathway in KN 65.2.

(1) Thi Phi Oanh, N.; Helbling, D. E.; Bers, K.; Fida, T. T.; Wattiez, R.; Kohler, H.-P. E.; Springael, D.; De Mot, R. *Applied Microbiology and Biotechnology* **2014**, *98*, 8235.
(2) Hashimoto, M.; Fukui, M.; Hayano, K.; Hayatsu, M. *Applied and Environmental Microbiology* **2002**, *68*, 1220.

P NOV 3

Looking for a perfect microorganism decomposing sodium dodecyl sulfate - environmental and genomic study

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Detergents due to their specific properties are widely used in household cleaning products, cosmetics and pharmaceuticals, thus, different surfactants are massively released to the environment. It is estimated that in 2015 world production of sodium dodecyl sulfate (SDS) will exceed 4 000 000 tons, what stands for approximately 30% of all detergents. SDS is known to be very toxic for aquatic organisms, and, due to its foaming properties and ability to increase adsorption of pollutants on solid particles, also impair the wastewater treatment. This results in elongation of wastewater purification process and significantly increases the costs. There are known technologies to eliminate detergents from production wastewater, however, industry is still looking for faster and more efficient ways for remediation. The most promising solution is to use microorganisms from habitats contaminated with this xenobiotic.
Question: We wanted to answer the questions: if microorganisms isolated from soil samples polluted with xenobiotics could degradate SDS and which genes are involved in the process?
Methods: Soil samples were collected from expired xenobiotic storage infrastructure in Poland. Bacteria were isolated by developed serial dilution protocol, where isolated strains were tested to grow on minimal medium with SDS as a sole carbon source. Rate of SDS degradation was verified using colorimetric test with "stains-all" reagent. Taxonomic identification of twenty most efficient SDS decomposers was determined by 16S rRNA sequencing. To identify genes involved in SDS metabolism whole genome sequencing of the most efficient strain was done followed by insertional mutagenesis and transcryptomic analysis.

Results: Almost 16% of the 700 isolated microorganisms were able to use SDS as a carbon source. The twenty most active strains were able to fully degradate 5 g/l SDS in less than 24 hours. 16S rDNA analysis confirmed that the strains belong to genus Pseudomonas. The isolate selected to the detailed analysis of genes and pathways required for SDS metabolism was able to grow even in a medium with 1% SDS.
Conclusions: Using classical microbiology approach we were able to isolate of culturable microorganisms with potential in SDS decomposition. Molecular biology technics and transcriptome profiling lead to better understanding of the whole process.

P NOV 4
Functional redundancy of multicomponent monooxygenases extend catabolic versatility in phenol- and toluene-degrading bacteria
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Aerobic bacterial degradation of phenol and toluene via ring hydroxylation is catalysed by several bacterial multicomponent monooxygenases (BMMs) characterised by wide substrate-specifity. The initial step in the biodegradation of phenol is the monohydroxylation reaction performed by multicomponent phenol monooxygenase (PH), while the degradation pathways for toluene involve either the oxidation of a methyl group by xylene/toluene monooxygenase coded by *xyl* genes of TOL pathway, or mono- (TOMO) or dioxygenation of the aromatic nucleus. In the indigenous isolates the co-expression of TOMO and TOL pathways have not been described yet. However, exceptionally, two different BMMs (TOMO and PH) have been described in the genome of *Pseudomonas* sp. strain OX1 where TOL pathway was initially silent, but was activated through a mutation, resulting in functional redundancy in this strain.
In our work the BMMs and its genes from 44 phenol- and toluene-degrading strains isolated from the Baltic Sea water were characterised. Among them the existence of two redundant pathways for toluene degradation (TOL and TOMO pathways) was shown in five *P. stutzeri* strains. Enzymatic activity of key enzymes, gene expression analyses by qRT-PCR and growth studies of the *touA* and *xylA* knockout mutants (the two genes code for the key enzymes of the two redundant pathways) indicated that in these indigenous strains both toluene degradation pathways are functional and expressing redundant catalytic functions. Both *touA* and *xylA* genes were inducible, revealing 7- and 16-fold-higher expression level with toluene comparing with that of uninduced cells. The whole genome sequence analysis of the redundant type strain 2A20 confirmed that the whole intact TOMO and TOL pathways are present in its genome.

P NOV 5
Map of the aromatic catabolic potential in the Roseobacter lineage
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Roseobacter is an ecologically important marine alphaproteobacterial group of metabolically versatile bacteria. The detection of the key genes for six central routes for catabolism of monoaromatic compounds (benzoyl-CoA, gentisate, homoprotocatechuate, phenylacetate, homogentisate and protocatechuate) in their genomes led to propose a role in aromatic hydrocarbon degradation in marine environments. Our aim was to determine whether roseobacters had all the genetic elements necessary for the six central catabolic routes previously mentioned plus catechol degradation. In addition, we included genes for several peripheral routes leading to these central pathways with the aim of generating a map of catabolic routes for aromatic compounds in roseobacters. We analyzed the presence of 115 proteins involved in central and peripheral catabolic routes for aromatic compounds in 43 genomes by checking the published annotation in the NCBI database and performing sequence homology searches in Roseobacter genomes using BlastP program. The more widespread pathways in Roseobacter were the protocatechuate branch of the β-ketoadipate, homogentisate and phenylacetate pathways, found in at least 50% of genomes analysed and putatively as complete routes. Furthermore, we confirmed that the meta-cleavage of protocatechuate is rarely found in roseobacters and we found that some roseobacters might have the catechol branch of the β-ketoadipate pathway. In addition, we report that roseobacters might degrade some peripheral compounds by two different pathways (i.e. benzoate degradation via benzoyl-CoA or via catechol), while other compounds are channeled only via one central compound (i.e. 4-hydroxyphenylacetate degradation via homogentisate). We observed variability in organization of pathway genes, with no clear relationship between gene order and Roseobacter

phylogeny. This study expands the catabolic potencial of Roseobacter towards aromatic compounds and provides a more complex view of the interaction between different peripheral pathways.

P NOV 6

Chlorobenzene Isotopic Fingerprinting analysis and microbial genetic profiling of natural consortia from a contaminated aquifer

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Compound Specific Isotopic Analysis (CSIA) is an emerging tool for the estimation of in situ biodegradation in groundwater. However, the degree of isotopic fractionation is strictly dependent on the enzymes involved in the different degradation pathways. For this reason, in case of compounds with multiple possible pathways, information about the actual microbial mechanisms involved in the degradation are necessary for a proper interpretation of isotopic data. In this work we propose the simultaneous use of CSIA and taxonomic/functional characterization of microbial populations as a set of tools to assess the rate and extent of biodegradation of organic compounds in contaminated sites under in situ conditions.
In the case-study site the groundwater is contaminated by different organic chlorinated compounds. Among them monochlorobenzene (MCB) has been selected as target pollutant. MCB has two different isotopic signature in the sites and, due to the different possible biodegradation pathways, this behavior could be ascribed to differences in both biodegradation pathways and contaminant sources. To disentangle the contribution of these processes we are carrying out an extensive taxonomic and functional characterization of the microbial communities at field and laboratory level. At the current stage of the project we set up microcosm experiments poising groundwater collected from the site both under oxygenated and anoxic conditions. In the microcosms, MCB degrading populations have been enriched by nutrient addition and multiple spiking of MCB. We described the structures of the enriched consortia and native site microbial communities through Illumina sequencing of 16S rRNA gene and we functionally characterised the communities by qPCR targeting reductive dehalogenase and dioxygenase genes putatively involved in MCB biodegradation.
Preliminary results showed that degradation of MBC occurred in both tested conditions amended with inorganic nutrients. Both microbial community structures and functional genes patterns actually differed between oxygenated and anoxic conditions, thus allowing the definition of specific molecular markers for aerobic and anaerobic biodegradation of MCB. Further investigation is ongoing to define new markers for degradation and couple these to the CSIA data.

P NOV 7

Microbiological Processes for the Degradationof Biodiesel in Groundwater

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The use of fossil fuels throughout the world has resulted in considerable environmental problems. Environmental and economic concerns associated with fossil fuels have encouraged the use of renewable transportation fuels, such as biodiesel. The increasing biodiesel demand could increase the probability of groundwater contamination as result of accidental and incidental spills during its production, transportation and storage. The applicability of existing groundwater biological treatment methods for biodiesel would depend on part on whether the indigenous microbial community can degrade these compounds or their chemically oxidized intermediates.The slightly oxidized metabolites resulting from chemical pretreatment should fit smoothly into existing biochemical pathways without novel functions required.A field experiment with a controlled biodiesel release (100 L by palm biodiesel) was conducted to assess the potential for bioremediation after the prechemical oxidation by using magnesium peroxide (MgO_2) and iron oxide. For groundwater analyses,samples were collected periodically from monitoring wells at depths 2, 3, 4, 5 and 6 m below ground surface. Both chemical (total organic carbon) and microbial parameters were measured throughout the study.The microbial community stimulated by the contamination and subsequent peroxidation reflects the preexisting microbial community. Differences between different parts of the site and different times indicated that the microbial community responded to the arrival of the biodiesel.

P NOV 8

Microbial diversity and function during different bioremediation strategies of diesel-polluted soil

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In numerous hydrocarbon-polluted sites, oxygen and pollutant bioavailability constitutes the main limiting factor for biodegradation because of the strong adsorption of hydrocarbons on organic soil particles (clay and peat). Therefore, several strategies such as biostimulation (with air/H_2O_2 and/or nutrients) or bioaugmentation are used, but often without understanding the endogenous microflora degrading capacity. This lack of differentiation between indigenous and added microorganisms could lead to poor predictability of the biodegradation efficiency. In addition, anaerobic degradation remains less applied in industrial settings for such compounds (especially for saturated hydrocarbons) as this process remains slow.
In this context, the main objective of our study was to understand how the bacterial community evolves, in terms of species and degrading gene diversities, during the application of three different bioremediation strategies in a heavily diesel-polluted clay soil: (i) anaerobic natural attenuation, (ii) bioventing and (iii) bioaugmentation with *Rhodococcus erythropolis* T902.1. In addition to the supply of new degrading genes, bioaugmentation with this biosurfactant-producing strain should facilitate the bioassimilation of desorbed hydrocarbons by the whole degrading microflora. This hypothesis is strengthened by previous results obtained during several microcosm- and pilot-scale experiments.
Aerobic and anaerobic microcosms were set up with three different soil samples coming from the same polluted site. Initially, their global organic content was identical but their hydrocarbon and peat concentrations were different, which led to differential oxygen consumption. Soils were sampled every 10 days to extract the DNA to measure changes in bacterial populations (with RISA analysis and 16S rRNA gene sequencing) and function (with qPCR and sequencing of degrading genes). Further analyses of the hydrocarbon content by GC-MS and of the genetic diversity by MiSeq metagenomic analysis provided detailed chemical and functional microbial data related to compound degradation and relative gene increases. Initial results showed significant differences in the microbial community structure. Moreover, *Rhodococci* seem to be maintained in the soil after inoculation.

P NOV 9

Biostimulation of anaerobic biodegradation by iron reduction processes in groundwater contaminated with a diesel/biodiesel blend (B20)

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Environmental and economic concerns about the use of fossil fuels stimulated the introduction of biofuels in world energy matrix. Today, many countries use different mixtures of biodiesel in commercial diesel. The increasing biodiesel use will increase the probability of groundwater contamination. Although biodiesel is commonly referred to as a harmless and readily biodegradable biofuel, the use of new diesel formulations contain BTEX (benzene, toluene, ethylbenzene and xylenes) require remedial action. Previous field experiments that evaluated the monitored natural attenuation of diesel B20 (20:80 v/v biodiesel and diesel) in groundwater demonstrated the development of strongly anaerobic conditions in the subsurface environment and indicated that the biodegradation of biodiesel slowed the BTEX degradation. Moreover, another experiment under fermentative methanogenic conditions in groundwater containing diesel B20 observed an increase of *Geobacter* spp, which was the main bacteria responsible for the iron reduction process. In this context, a controlled field experiment has been conducted to assess the potential for iron reducing conditions (by iron oxy-hydroxide recovered from acid mine drainage) to enhance biodegradation BTEX in groundwater contaminated with diesel B20. Physical chemical analysis (pH, redox potential, concentration of nitrate, total iron, sulfate, acetate, nitrite, ferrous iron, sulfide, methane, BTEX) and microbial analysis (ribosomal *rRNA intergenic spacer analysis* (*RISA*)**,** polymerase chain reaction (PCR) and metagenomic sequencing) were used to assess the biogeochemical and microbial community structure changes as well as to determine the genes involved in degradation processes.

P NOV 10

Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) degradation ability in Fosmid library from oil-impacted mangrove sediment

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Mangrove is a biome that promotes the maintenance of life in the seas and balance of the biosphere. The importance of this ecosystem is due to its great biological productivity and it is found in estuaries, deltas of rivers, lakes and islands, serving as shelter for several species of fish, crustaceans, molluscs, birds, reptiles, mammals and microorganisms. One of the main interests of science is to find bioactive compounds, which holds relevance to society. To achieve this goal, many genetic researches are being done in different environments around the world. However, approximately 99% of soil microbiota cannot be isolated by cultivation methods. In this context, cultivation-independent methods, such as "metagenomics", are needed for a greater knowledge and exploitation of microbial diversity. Metagenomic libraries are a powerful tool to access the sediment microbial diversity. Mangroves have suffered the most diverse human activities with economic and agricultural purposes, fishing and waste disposal, among others. One of the Brazilian mangroves located in Bertioga city, São Paulo state, was affected by an oil spill occurred in the 80s, which affected the health of various species of fauna, flora and microbiota. Currently, this region still feels the consequences of this disaster, which led to the study of biotechnological potential of their microorganisms. Therefore, this study aimed to exploit a metagenomic library constructed from mangrove sediments in the search for degradation ability of diverse Polycyclic Aromatic Hydrocarbons (PAHs). The library was previously constructed using the "Cloning pCC2FOS-Ready Copy Control" kit, following the manufacturer's methodology. A total of 12,800 clones was obtained, of which 2,880 were screened for PAHs degradation activity. These clones were initially grown in LB culture medium with chloramphenicol (12.5 µg/ml) and L-arabinose (0.02%), and then inoculated in BH (Bushnell Haas) culture medium containing, as the only carbon source, one of the following PAHs: phenol, naphthalene, phenanthrene, pyrene and benzopyrene. After 48 hours at 37 ° C and 180 rpm,Thiazolyl Blue Tetrazolium Bromide(MTT) was added as a colorimetric indicator of cell growth. Forty eight clones showing degradation activity for at least one of the five tested hydrocarbons were detected. These results show the great potential of this metagenomic library for investigation of new genes coding for enzymes involved in PAHs degradation pathways.

P NOV 11

Microbial community responses to contamination in mangrove sediment as revealed by metatranscriptomics

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Anthropogenic activity has greatly contaminated mangroves areas, which have suffered the most diverse human activities with economic and agricultural purposes. The microbial community structure is a sensitive indicator of changes in the ecosystem. Here, we collected sediment samples from polluted and pristine mangroves and determined the expression of functional genes involved in toxic compounds resistance (i.e. Cu, Zn, Cd, Pb, Hg and antibiotics) using metagenomic and metatranscriptomic approaches. Three different mangrove sampling sites on the coast of São Paulo State, Brazil were chosen: 1) highly oil impacted area (Oil Mgv); 2) moderately impacted area by anthropogenic activity (Ant Mgv) 3) Pristine area (Pristine Mgv). From each of these, core sediment samples were collected for DNA and RNA extraction. Libraries were sequenced using Illumina® HiSeq 2000 platform and the resulting sequences were uploaded into the MG-RAST server (http://metagenomics.anl.gov). The statistical assessment was performed using Statistical Analyses of Metagenomic Profiles (STAMP). The metatranscriptome results indicated a significant abundance of genes involved in *czc* system (mediates resistance to cobalt, zinc and cadmium) and efflux system for resistance to copper and silver. *Cobalt-zinc-cadmium resistance protein CzcA*; *Cobalt-zinc-cadmium resistance protein, Cation efflux system protein CusA* were highly expressed in Oil Mgv and Ant Mgv sites (p-value <1 e^{-15}; 4.99 e^{-9}; 8.66 e^{-9}; 5.42e^{-8}, respectively). Some genes involved with drug resistance in Ant Mgv site, for example: *Acriflavin protein* were also highly expressed (p-value <1 e^{-15}). On the other hand, in the Pristine area, heavy metal resistance was not highly expressed, while some genes involved with drug resistance were, for example: *DNA gyrase subunit A and B (EC 5.99.1.3)* and *Acriflavin protein* (p-value <1 e^{-15}). In conclusion, the high

expression of genes involved in metal resistance in Oil Mgv area, suggests the occurrence of contamination by oil in the past. It is hypothesized that contamination was able to interfere in the microbial community functioning in mangroves. Financial Support: FAPESP Process nº: 2011/50809-5; 2012/16850-0; 2013/20670-0.

P NOV 12

Deciphering the roles of the members of a bacterial consortium in the degradation of thiabendazole: combining SIP-DGGE with meta-omics

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Question: Thiabendazole (TBZ) is a highly persistent fungicide, used in the post-harvest treatment of fruits. The reduced efficacies of expensive wastewater treatment strategies in removing TBZ from effluents render it an ecological threat. The use of microbial inocula for TBZ waste treatment seems to be an economically viable solution, but no effective biodegrading agent has been identified to date. The aim of the present study was to isolate bacteria able to rapidly degrade TBZ and to study the associated mechanisms.
Methods: Enrichment cultures and extended sub-culturing were used for the isolation of a stabilized TBZ-degrading consortium. Analytical and molecular methods were used to monitor the degradation of TBZ and the microbial community composition. Stable isotope probing - DGGE and qPCR were used to identify the key bacterial component responsible for TBZ degradation. Metagenomics were employed to obtain the full picture regarding the composition of the consortium and to assess the genetic mechanisms driving the metabolism of this fungicide.
Results: The consortium rapidly degraded TBZ at concentrations reaching 750 mg L^{-1} in liquid cultures and 500 mg kg^{-1} in soil. Metagenomics analysis resulted in 51,109 contigs with a total length of ~99 Mbp. Analysis of contig residing phylogenetic marker genes revealed a consortium comprising *Bacteroidetes* (*Flavobacterium* sp.), α-*Proteobacteria* (*Sphingomonas* and *Rhizobiales*), β-*Proteobacteria* and γ-Proteobacteria. Pathway analysis demonstrated high versatility in terms of aromatic compound degradation. SIP-DGGE and qPCR suggested a key role of a *Sphingomonas* in the TBZ degradation.
Conclusions: Successful enrichment of a TBZ-degrading consortium, with *Sphingomonas* being central in this process. On-going Metatranscriptomics/ metaproteomics analysis is expected to elucidate associated knowledge gaps.

P NOV 13

Nickel and Copper biosorption by EPS producing *Ensifer adhaerens* strain As3-5a

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Anthropogenic and industrial activities are responsible for environment contamination through the release of toxic heavy metals. Recent studies have reported that microbial biofilms producing extracellular polymeric substances (EPS) contribute significantly to heavy metals removal due to the capacity of EPS to bind and sequester heavy metals from industrial effluents.
In the present work, an EPS producing bacterial strain, affiliated to *Ensifer adhaerens*, was characterized and used in biosorption experiments, in order to implement Nickel and Copper removal process from electroplating wastewaters.
Ensifer adhaerens was grown in Luria Broth (LB) medium in a 5L bioreactor. At defined sampling times (24, 48 and 72 hours), cultural broths were deposited onto cellulose acetate membranes. Nickel (50 mg/L) and Copper (200 mg/L), provided as distilled water solutions as well as electroplating wastewaters, were passed through the biomass-activated filters. Abiotic systems were also prepared in order to monitor abiotic losses of heavy metals. Nickel and Copper analysis were conducted by inductively coupled plasma mass spectroscopy (ICP-MS). Twenty four hours-grown biomass removed from water solutions 6.12 mg/L of Nickel and 132.17 mg/L of Copper, separately. When both present in bimetallic water solution, the two metals were removed more efficiently: Ni 25.15 mg/L and Cu 174.03 mg/L. When electroplating wastewater was passed through the biomass-activated filter the removal of Nickel was 17.56 mg/L in the absence of Copper, and of 22.48 mg/L of Nickel and 204 mg/L of Copper in the presence of a bi-metallic wastewater. When the biomass was grown in EPS not inducing conditions, heavy metal removal was not observed, suggesting their role in the process.
The high biosorption potential of *Ensifer adhaerens* strain As3-5a in single and bi-metal systems indicates that the EPS producing strain may be exploited as eco-friendly and low-cost biotechnology for the clean-up of industrial effluents from nickel and copper.

Keywords: EPS, nickel, copper, bioreactor, bioremediation.
Acknowledgements: The financial support by MIUR-Regione Lombardia contract No. 30066464 was greatly appreciated.

P NOV 14

Assessment of anaerobic biodegradation of polyvinylchloride by enriched marine communities

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Persistence of petroleum-derived plastics in the environment resulted in their accumulation in huge amounts leading to environmental and health concerns. Specifically, polyvinylchloride (PVC) is one of the most produced polymers for which the conventional management practices have been reported to result in the production of toxic compounds. Microbial biodegradation could be a safe and eco-friendly approach for safe management of the plastic waste. To date there are very few reports on the biodegradation of this synthetic polymer by fungi while no studies have been performed on the fate of PVC entering the marine environment. The aim of this work was to assess the ability of anaerobic Med Sea-dwelling microbes to degrade virgin PVC film. This was performed by enriching anaerobic microbial communities from marine samples collected in Aegean Sea, in the presence of the plastic films as carbon source and different electron acceptors. The microbial growth was monitored by quantification of the proteins adhered to film surfaces and measuring consumption of electron acceptors and gas production. The PVC film biodegradation was evaluated by thermogravimetrical analysis (TGA), gel permeation chromatography (GPC) and gravimetric measurements.
After 7 months of incubation, growth of microbial communities was observed in all enriched consortia. TGA showed lower thermal stability of the PVC films incubated with 5 different communities. After 10 months of incubation, a weight loss percentage of up to 12% was observed in four consortia. PVC polymer chain biodegradation was confirmed by GPC from 1 consortium enriched under nitrate-reducing conditions.
The results highlight that anaerobic biodegradation of PVC films occurred in the presence of enriched marine anaerobic communities. Microbial communities analysis is in progress to identify the microorganisms involved.

Acknowledgements: The financial support by FP-7 project BIOCLEAN contract No. 312100 is greatly appreciated.

P NOV 15

Inferring oxybenzone biodegradation at low concentrations through bacterial biomass measurements.

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Controversial issues have emerged in the last few years about the nature of oxybenzone, a widespread component of sunscreens and skin care products. It is mainly used as a broad-spectrum UV filter and photoprotective agent. Concerns have been expressed about the potential of oxybenzone to be an endocrine disruptor, coral-bleaching inducer, penetration enhancer and about its correlation with endometriosis.
The objective of our study was to test the ability of the lake bacterial community to degrade oxybenzone. We used a flow cytometry-based method developed in our lab to assess the chemical compound's biodegradation by measuring the increase of prokaryotic community size over time with direct cell counting. The method is based on the assumption that biomass can only increase through metabolization of the target molecule's carbonic part, assuming that it is the single available Carbon source in the medium. A significant bacterial growth in oxybenzone medium has been measured and confirmed by Microdish cultures on oxybenzone-silicate medium. Three strains capable of degrading the chemical were isolated and identified (i.e. *Acinetobacter johnsonii, Acinetobacter tjernbergiae and Pseudomonas migulae)*.
The enrichment of the oxybenzone bacterial community composition and diversity over 3 weeks was determined by Illumina sequencing. High Performance Liquid Chromatography was used in order to establish the amount of oxybenzone degraded over 48 hours. Further experiments are being performed in order to determine the likelihood of degradation of oxybenzone into benzophenone-1. At this point of the study it appears that *A. johnsonii* and *P. migulae* might take this degradation pathway whereas *A. tjernbergiae* uses another one currently unknown.

Oral presentations

O SYMI 1

Functioning of lichen symbioses is supported by a diversified bacterial microbiome

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Lichen habitats comprise environments with considerable changes in humidity, temperature, irradiation and nutritient availability. Nonetheless, many lichen species persist for remarkable time periods under hostile conditions and recover the functionality of the symbiotic partners after rehydration. We have explored the structure of the lichen microbiome and its functional contributions to the symbiosis, using the lung lichen *Lobaria pulmonaria* as the model. We found that bacterial communities contribute multiple aspects to the symbiotic system, including essential functions such as i) nutrient supply, especially nitrogen, phosphorous and sulfur, ii) resistance against biotic stress factors (*i.e.* pathogen defense), iii) resistance against abiotic factors, iv) support of photosynthesis by provision of vitamin B_{12}, v) fungal and algal growth support by provision of hormones, vi) detoxification of metabolites, and vii) degradation of older parts of the lichen thallus [1]. The antagonistic potential providing resistances against biotic stress factors can be used for various biotechnological applications, *e.g.* for sustainable agriculture. Lichen-associated *Stenotrophomonas* strains were found to be the most active antagonists in the cultivable microbiome. These isolates were also shown to produce and secrete feasible amounts of stress-protective metabolites (*e.g.* spermidine) *in vitro*. Furthermore, selected strains used for seed inoculation demonstrated growth promotion in *Solanum lycopersicum* L. under limited irrigation.

[1] Grube *et al*. (2014) ISME J 9: 412—424.

O SYMI 2

Biological activity and colonization pattern of the beneficial endomycotic bacterium *Rhizobium radiobacter* F4 in plant roots

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Rhizobium radiobacter F4 (*Rr*F4) was originally isolated from the growth-promoting fungus *Piriformospora indica* that forms a tripartite Sebacinalean symbiosis with wide range of host plants (Sharma et al., 2008). The genome of *Rr*F4 was fully sequenced using 454 pyrosequencing and the draft genome showed very high synteny with the fully annotated genome of *Rhizobium radiobacter* (formerly: *Agrobacterium tumefaciens* C58; the sequence of the single plasmid of each strain showed however deletions and other differences. Here, we show that plants colonized by *Rr*F4 increased biomass and enhanced systemic resistance against the bacterial pathogens *Pseudomonas syringae* pv. *tomato* DC3000 in Arabidopsis and *Xanthomonas translucens* pv. *translucens* in wheat, respectively. Quantitative real-time PCR analyses confirmed the proliferation of *Rr*F4 in roots of axenically grown barley, wheat and Arabidopsis. GUS and GFP-tagged *Rr*F4 were used to study the colonization pattern of *Rr*F4 in roots using light, confocal laser scanning microscopy and raster and transmission electron microscopy.
*Rr*F4 mainly colonized the root hair zone forming dense biofilms at the root surface. The emergency side of root hairs and lateral root protrusions were identified as distinct entry sides into the root tissue. Unlike its fungal host, *Rr*F4 colonized not only rhizodermis and cortex tissue but progressed beyond endodermis into the stele. This results show for the first time a detailed insight into the localization of a Sebacinales derived plant-beneficial bacterium inside the roots of mono- and dicotyledonous host plants.

Reference:
Sharma, M., Schmid, M., Rothballer, M., Hause, G., Zuccaro, A., Imani, J., Schäfer, P., Hartmann, A., Kogel, K.-H. (2008) Detection and identification of mycorrhiza helper bacteria intimately associated with representatives of the order Sebacinales. Cell. Microbiol. 10, 2235-2246.

O SYMI 3

Fate of pathogenic *Bacillus cereus* spores after ingestion by protist grazers

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The aim of this study is to understand the symbiosis between bacterivorous protists and pathogenic bacterial spores, in order to gain insight on survival *and dispersal* of pathogenic bacteria in the environment. It is generally accepted that resistance to grazing by protists has contributed to the evolution of *Bacillus cereus* group bacteria (e.g. *B. cereus*, *B. anthracis*, *B. thuringiensis*) as a pathogen. It has been hypothesized that the spore stage protects against digestion by predating protists. Indeed, *B. thuringiensis* spores have been shown to be readily ingested by ciliated protists but failed to be digested (Manasherob et al 1998 AEM 64:1750-).
Here we report how diverse protist grazers grow on both vegetative cells and spores of *B. cereus* and how the bacteria survive ingestion and digestion, and even proliferate inside the digestive vacuoles of ciliated protists. The survival ability of *B. cereus* was initially investigated in microcosms inoculated with pure cultures of the protists *Acanthamoeba castellanii, Tetrahymena pyriformis* and *Cercomonas* sp. as grazers. Individual protist cultures were fed with fluorescently labelled (CellTracker™RedCMTPX) *B. cereus* spores or vegetative cells as the only food source. The presence of fluorescently labelled intracellular bacteria confirmed that *B. cereus* spores as well as vegetative cells were ingested by protists and appeared intact when observed by epi-fluorescence microscopy. Secondly, *B. cereus* digestion and protist growth were determined by qPCR and protists appeared to grow on spores, though they grew better on vegetative cells. Finally, *B. cereus* spore germination was observed inside the ciliated protist *T. pyriformis* after antibiotic treatment of the protist surface which seems contradicting to the observed protist growth on spores. Initially these observations indicate that protists might act as a survival niche and potential breeding ground for *B. cereus* with some loss of bacteria to support growth of the protist. This indicates tight symbiosis between bacteria and protist grazers and will be discussed.

O SYMII 1

Meeting the aliens: morphogenesis induction of the green alga *Ulva mutabilis* by sponge-associated bacteria highlights functional redundancy as a key factor shaping marine holobiont communities

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It is often assumed that symbiont microbial communities function as a fitness-enhancing factor to the benefit of their eukaryotic hosts. Regardless of their nature, the presumed benefits would suite the physiological and metabolic demands of the host organism, suggesting that specialization is a key feature of fine-tuned, host-associated microbiomes. Here, we challenge this contention and test whether symbiont bacteria are capable of triggering positive physiological responses in an exogenous, non-corresponding host organism. This was achieved by exposing axenic gametes of the green alga *Ulva mutabilis* to marine sponge bacterial symbionts using a morphogenesis-induction bioassay in controlled microcosms. The development of the gametes into germlings was monitored in the presence/absence of sponge symbionts and in co-cultivation with *Ulva*-specific bacteria after 18 and 42 days of incubation. We observed that sponge-derived bacteria in the *Rhodobacteriaceae* clade (*Alphaproteobacteria*) - including *Pseudovibrio*, *Ruegeria* and so far unclassifiable strains - were inexorably capable of promoting the morphogenesis of *Ulva* at varying degrees, a phenomenon believed to be carried out exclusively by indigenous symbionts of the alga. Genome sequence mining indicates that the biosynthesis of signaling molecules such as indole and its derivatives emerges as an overriding feature underlying these effects. However, most sponge-associated bacteria could not induce complete algal morphogenesis alone, but in the presence of an *Ulva*-specific *Maribacter* symbiont. In-depth 454-pyrosequencing community profiling and phylogenetic inference demonstrated that the sponge symbionts used in our experimental manipulation did not show taxonomic overlap with the typical *Ulva*-associated bacteria. Therefore, we report on a readily identifiable, measurable function of crucial relevance to host development that can be elicited by invading symbionts showing no tight co-evolutionary relationships with the recipient organism. We conclude that benefits derived from allochthonous bacterial metabolism might play an important role in algal morphogenesis, highlighting generalist microbial traits as overriding factors structuring eukaryotic symbiont communities in the seas.

O SYMII 2

Nitrogen-fixing symbioses between unicellular organisms are critical for open ocean ecosystems

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Introduction: Nitrogen fixation is a critical source of fixed nitrogen for open ocean oligotrophic gyre ecosystems. Historically, nitrogen fixation was believed to be primarily due to the filamentous cyanobacterium *Trichodesmium*. Flow cytometry and metagenomics led to the discovery that the uncultivated, widespread nitrogen-fixing unicellular UCYN-A cyanobacterium is unusual, lacking photosystem II, Rubisco and the entire TCA cycle. Single-cell sorting and metagenomic techniques showed that the cyanobacterium with a 1.4 Mb streamlined genome, is symbiotic with a small single-celled alga, the haptophyte *Braarudosphaera bigelowii* and that it retains photosystem I. It is now known that there are divergent, but related strains with the same metabolic deletions, and it is unclear how this simple single-celled symbiosis functions, and how nitrogen fixation is supported in the cyanobacterium.
Objectives: The objectives of this study were to determine the diversity of the symbiosis, and if PSI is used by UCYN-A to support nitrogen fixation.
Materials & methods: Flow cytometric sorting, RT-PCR, and whole genome microarrays, were used to interrogate natural populations of UCYN-A for PSI gene expression, whole genome expression, and microbial community metatranscriptome expression.
Results: Results show that there are genetically distinct clades of UCYN-A (average ~80% nucleotide identity). PSI is expressed in two UCYN-A strains in a pattern inversely related to that of nitrogenase. Whole genome expression shows that some genes are expressed during the light along with nitrogenase, indicating they may be involved in donating electrons for nitrogen fixation.
Conclusions: UCYN-A expresses photosystem I genes in a daily cycle inverse to that of nitrogenase expression and photosystem I may be important in balancing energy and electron flow.

O SYMII 3

Nutritional complementation in an insect-bacterial symbiosis: un ménage à trois

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Although one bacterial symbiont is sufficient to meet the nutritional needs of some insect hosts, other hosts appear to require two or more bacterial partners with different metabolic capabilities. Even though the associations involving more than one bacterial partner are hypothesized to have arisen from genomic deterioration of the symbionts, the wider question of how the metabolisms of the different partners are integrated remains to be established. We investigate this problem in an association that thrives under conditions of nutritional scarcity. Our experimental system is formed by a spittlebug and its bacterial symbionts. Our strategy is to use genomic meta-transcriptomic data in order to reconstruct metabolic models for the three symbionts and their host. The results obtained thus far confirm that *Sulcia* and *Sodalis* are the principal suppliers of essential amino acids (EAA) to the host. The analyses of the data show that pathways for EAA can be divided into 3 categories: i) pathways present exclusively in Sulcia; ii) pathways present exclusively in the Sodalis-like bacterium; iii) pathways present in both symbionts. The results obtained are important in order to understand the interactions between the different symbionts and their host inside the bacteriome.

O SYMII 4

***Spiroplasma*, a new symbiont in tsetse flies**

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Introduction: Tsetse flies, the primary vectors of African trypanosomes, have established symbiotic associations with diverse bacterial species. Tsetse flies have been reported to harbor up to three symbionts - obligate *Wigglesworthia*, commensal *Sodalis* and *Wolbachia*, which is mainly known for its ability to induce reproductive alterations in its arthropod hosts.
Objectives: In this study, we examined the presence of *Spiroplasma* in tsetse flies. We surveyed *Spiroplasma* infection prevalence in more than 320 individuals from 10 different *Glossina* species. The infection density was quantified and the

Spiroplasma strain was tissue localized. The enhanced knowledge of this new symbiotic association may provide useful information for controlling this devastating disease vector.

Materials & methods: The infection of *Spiroplasma* was examined in a total of 327 specimens of tsetse flies using a *Spiroplasma* specific *16S rRNA*-based PCR assay. Genotyping was performed on a total of seven genes markers. *Spiroplasma* density was quantified by qPCR using *dnaA* gene specific primers and normalized to the host gene *β-tubulin,* from gut and reproductive tissues. Localization of *Spiroplasma* in *Glossina fuscipes fuscipes* was performed by FISH using specific fluorescent probes.

Results: *Spiroplasma* infections were detected in 54 samples out of 327 (16,5%) and it was found in three out of the ten analysed *Glossina* species. The phylogenetic analysis indicated that tsetse flies harbour very closely related *Spiroplasma* species belonging to the citri clade of *Spiroplasma*. Based on qPCR results, *Spiroplasma* density was significantly higher in the male than the female tissues, as well as in the gut of larvae than the next developmental stages of both genders. Finally, using FISH we were able to tissue localize *Spiroplasma* in tsetse flies.

Conclusions: Detection and characterization of a *Spiroplasma* strain in tsetse flies was performed. The bacterial density indicated that in lab colonies *Spiroplasma* is not evenly distributed between male and female adults.

O SYMII 5

Honeybee symbionts protect their host from American Foulbrood disease

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Recent mass declines, associated to both abiotic and biotic factors, are severely affecting pollinator insects. Despite intense research on the microbial communities associated to the pollinators, little is known about the contribution of gut symbionts to honeybee health.

Here we show that selected gut symbionts have a beneficial effect on the larvae by efficiently protecting from *Paenibacillus larvae*, the causative bacterium of the American Foulbrood Disease, one of the most devastating diseases of the beehive. We characterized the bacterial microbiome associated to larvae of symptomatic and asymptomatic beehives using multiple approaches targeting 16S rRNA gene including PCR-DGGE, phylochip and 454-based high throughput sequencing. A gut dysbiosis was observed in individuals from symptomatic beehives that were dominated by *P. larvae* signatures, while asymptomatic animals presented the typical assembly already associated to healthy larvae. Following an intensive screening of bacterial isolates from asymptomatic healthy larvae, we selected two spore-forming bacteria capable of inhibiting *P. larvae* growth *in vitro*. Both the strains and their combination reduced the larvae mortality *in vivo* following exposure to the pathogen. Such a protection was confirmed in the beehive during field experiments. In the beehive, the larvae treated with the strains were protected from *P. larvae* infection with the combined treatment of the two strains being the most effective. The protection was contributed by multiple controlling factors including direct antagonism against *P. larvae*, elicitation of the expression of antimicrobial peptides and competitive exclusion of the pathogen due to the colonization of the gut epithelium as shown by fluorescent *in situ* hybridization.

Poster presentations

P SYM 1

Enrichment experiment reduces diversity and changes microbial interactions in an ultra-oligotrophic environment

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The increase of nutrients in water bodies, in particular nitrogen and phosphorus due to the expansion of agricultural and other human activities is accelerating environmental degradation, elevating the risk of eutrophication and reducing biodiversity. To evaluate the ecological effects of the influx of nutrients in an oligotrophic and stoichiometrically imbalanced environment, we performed a replicated *in situ* mesocosm experiment. We analyzed the effects of a N- and P-enrichment on the bacterial community structure and especially on various features related to the diversity and nature of interspecific interactions. We focused on the biofilm formation and antibiotic resistance of 960 strains of cultivated bacteria in two habitats, water and sediment, before and after three weeks of fertilization. The experiment was conducted in the Cuatro Cienegas Basin in Mexico, a desert ecosystem comprised of several aquatic systems. The abundance of nutrients in this basin exhibits strong stoichiometric imbalance, suggesting that species diversity is maintained towards competition for resources. The water habitat was dominated by *Pseudomonas,* while *Halomonas* dominated the sediment. A significant loss in species richness was observed after nutrient enrichment. Strong antibiotic resistance was found among the isolates at time zero in the nutrient-poor bacterial communities, but resistance declined in the bacteria isolated in the nutrient-rich environments, suggesting that in the nutrient-poor original environment, negative inter-specific interactions were important, while in the nutrient-rich environments, competitive interactions are not so important in relation to adaptations that favor rapid growth using new and abundant resources. In water, a significant increase in the percentage of biofilm-forming strains was observed for all treatments involving nutrient additions. However, in sediment, differences between before and after the treatments were not significant. When this diverse and fragile bacterial network of interactions was perturbed by the mesocosm conditions and the nutrient input, the community structure changed, reducing its overall diversity, suggesting that the resilience of this extremely oligotrophic oasis depends precisely on the permanence of such unbalanced stoichiometry.

P SYM 2

SYBR Green I based quantative real-time PCR assay for removal rate of adenovirus as surrogate of norovirus using polyolefin microfilter membrane

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Question: Noroviruses (NoVs) are positive-sense single-stranded RNA viruses that belong to the Caliciviridae family. NoVs are now the leading cause of gastroenteritis in the world. But, it is difficult to detect them because they are infected at very low level and cannot be grown in cell culture. Therefore viral surrogates are widely used by researchers. In this study, to evaluate the removal rate of NoVs using micro-filter membrane (MF), we used Adenoviruses (AdV) as a surrogate model.
Methods: The goal of the present study was to estimate the removal rate of AdV using polyolefin MF by quantitative real-time PCR using SYBR Green. To evaluate the removal rate of AdV, we used a polyolefin MF which has an effective area of $0.01m^2$ and then 4 $x10^8$ PFU/ 2L (plaque forming units) were spiked with known titer of AdV. The water samples were collected as follows. First, only raw water as negative control sample was collected. Second, 4 x 10^8 PFU of viruses were spiked with distilled water and raw water, respectively. After fully mixed, we collected the distilled water with virus spiking sample and raw water with virus spiking sample. After spiked with 4 $x10^8$ PFU of viruses, each water sample was passed through the polyolefin MF and collected. After the pre-treatment of distilled water with virus and raw water with virus, finally the supernatants were passed through the polyolefin MF and they were collected. To generation of quantification standards, AdV PCR product was cloned into PCR2.1-TOPO vector.
Results: The concentration of the plasmid DNA was 121.7 ng/uL, which equates 2.66 x 10^{10} copies/uL. A series of 10 fold dilution starting from 1 x 10^{10} to 1 x 10^0 were prepared. The standard curve plot has a slope of -3.025, coefficient of determination of 0.965 and a reaction efficiency of 114.087%. The lower detection limit was 1 to 100 copies containing only raw water. The copy number of Adv in distilled water and raw water with virus ranged from 10^7 copies/uL. The copy number of Adv in distilled water, raw water with virus passed through the MF samples ranged from 10^1~10^3 copies/uL.

Conclusions: No difference was observed regardless of water quality. The samples with passed through the polyolefin MF showed greatly reduced copy number of Adv. Finally, Adv is suitable for removal rate of NoVs using MF as a surrogate model.

P SYM 3

New unexpected functions for ACC deaminase genes in the *Sinorhizobium meliloti*

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Mutualistic cooperation is one of the most fascinating issue in evolutionary biology and legume-rhizobia symbiosis represent models of cross-kingdom mutualism. However, not all strains of the same rhizobial species have the same mutualistic phenotype, specifically they show different symbiotic performances and up to now only a few studies addressed the genetic basis of these differences (Galardini et al. 2011). In this context one of the most intriguing gene is that encoding the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (*acdS*) present in the dispensable genome of the model species *Sinorhizobium meliloti.* AcdS is supposed to be involved in the sequestering and cleaving of plant-produced ACC, the precursor of the plant stress hormone ethylene (Glick 2005). However, the function of *acdS* in symbiotic bacteria has not been fully clarified, especially in relation to the mutualistic behavior of rhizobial strains. Indeed,few data are available on the effect of such gene in symbiotic competitiveness (Ma et al. 2004) and then on the selective benefit it may confer. To clarify this issue, an extensive phylogenetic and comparative genomic analysis of *acdS* orthologs was performed in genomes of *S. meliloti* strains and functional studies were carried out by expressing *acdS* from natural strains, in the model strain *S. meliloti* Rm1021, which lacks *acdS* gene. Then, the symbiotic and endophytic phenotypes of recombinant vs the parental strain were evaluated with respect to competition for root nodule occupancy, plant colonization and modulation of ethylene production by the host plant. Additionally, phenotype microarray experiments were performed to investigate the metabolic function carried out by AcdS.Data showed that the *acdS* orthologs present in different *S. meliloti* strains are polyphyletic and may indeed derive from different alphaproteobacteria representatives. No increase in fitness for nodule occupancy was found in the Rm1021 strain expressing *acdS* compared to the parental one, as well as faint effects on the modulation of plant ethylene levels were observed. Surprisingly, AcdS was shown to confer the ability to utilize formamide and some dipeptites as sole nitrogen source. We conclude that *acdS* in *S. meliloti* could be more related to the exploitation of unusual nitrogen sources, in connection with rhizospheric colonization or endophyitic life-style (Pini et al. 2012) than to the symbiotic interaction.

P SYM 4

The utilization of a degrading and a probiotic consortium of bacteria to improve the health of corals following an oil spill

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Coral reefs are among the most important, but also the most endangered ecosystems in the world. Several effects of anthropic activities are involved in this threat, such as global warming and chemical pollution. In this context, the impact of oil spill on corals and their microbiota is not well described. In addition, the most used solution in cases of oil spills is the application of chemical dispersants, which may have a more harmful effect in corals than that of the oil itself. In light of this, the aim of the present study was to develop an efficient strategy for bioremediation of coral reefs by using oil-degrading and possibly probiotic microorganisms isolated from the scleractinian coral *Mussismilia harttii*. The experiment was performed in a microcosms where corals were subjected to the following treatments: control (seawater only); bioaugmentation (bacterial consortium); oil + bioaugmentation (bacterial consortium and oil); oil (oil). After 10 days of treatment, the following parameters were measured: degradation capacity of the microbial consortium; photosynthetic capacity of zooxanthellae

associated with corals; response of molecular biomarkers; and changes in the microbial community associated with corals, using DGGE and next generation sequencing of 16S rRNA gene. Results showed a higher rate of oil degradation in the presence of the bacterial consortium, as well as an attenuation of the oil impact on corals. The observed improvement in corals health in the presence of the consortium was likely due to a high efficiency of the degradation and probiotic activity of the consortium. The taxonomic analysis showed a recovery of the native bacterial community of the coral holobiont in the presence of the consortium. Among the species recovering the native bacterial community, we highlight a member of the consortium classified as *Vibrio alginolyticus,* a probiotic bacterium used as a biological control agent in aquaculture. These results demonstrate that the methodology proposed in the present study is promising, and can help in increasing survival of coral reefs following an oil spill.

P SYM 5

Sugar cane bagasse affects bacterial community dynamics in the sheep rumen

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Ruminants are herbivores and have evolved a symbiotic host-microbe relationship with a complex microbial community inhabiting the rumen allowing the use of lignocellulosic biomass as their main energy source. Considering that diet is one of the main drivers shaping the structure of the rumen microbiome, we investigated the impact of sugarcane bagasse in the rumen bacterial community dynamic using 16S rRNA (V3 and V6 regions) amplicon sequencing. We assessed three rumen-cannulated adult male sheep (*Ovis aries*) fed on a diet consisted of 30% concentrate and 70% roughage (control treatment) and three sheep fed on the same diet, but with 14% of the roughage portion replaced by sugarcane bagasse. Fluid and fiber were separately sampled 3 hours and 15, 30, 45, and 60 days after starting the experiment. Total genomic DNA was extracted from 60 independent samples (2 treatments X 3 replicates X 5 time points X 2 types of samples, i.e. fluid or fiber) for downstream analysis. The DNA was used as a template for amplification of V3 and V6 regions of the bacterial 16S rRNA gene and then sequenced using the PGM™ (Ion Torrent). Overall, the two dominant phyla were Bacteroidetes (42%) and Firmicutes (37%). The most abundant bacterial genus was Prevotella (20%), followed by Clostridium (9%), Ruminococcus (8%) and Butyrivibrio (2%). The principal coordinate analysis (PcoA) showed that the bacterial community was significantly different in both treatments at 60 days. Bacteroidales, Actinomycetales and Clostridiales were the top dynamic bacterial orders that significantly increased in relative abundance in the treatment with sugar cane bagasse after 60 days. Canonical correspondence analysis (CCA) revealed that the Clostridiales and Bacteroidales are positively correlated with propionate, butyrate, ammonia, and pH. These results indicate that a small replacement in the diet roughage portion influences de dynamic of specific bacterial taxa. This strategy can be used to reshape the bacterial community in the sheep rumen aiming to enrich the targeted bacterial taxa. Support FAPESP 2012/03848-8, 2012/24588-4 and 2014/00448-4.

P SYM 7

Do Different Coral Species Represent Different Biotechnological Sources?

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Corals harbor diverse symbiotic microorganisms that are indispensable for their survival. This phenomenon makes corals highly relevant for the discovery of enzymes that are of biotechnological interest. However, it remains unclear whether various coral species in the same region possess distinctive biotechnology potentials. To address this question, the coral species *Mussismilia braziliensis*, *Millepora alcicornis* and *Porites astreoides* were collected in the same region, Recife de Fora Reef, Brazil. The abundance, similarities, and enzymatic production of coral microbial communities were evaluated. No differences in the abundance of bacteria were observed. However, the bacteria, archaea and micro-eukaryote community structures indicated specific associations for each species studied, corroborating the microbiota host specificity hypothesis. Forty-nine bacterial colonies were isolated, and DNA sequencing revealed a high diversity of cultivable microbes dominated by *Exiguobacterium, Bacillus, Halomonas* and *Staphylococcus.* Eight isolates were candidates for new species descriptions. Enzymatic assays showed that isolates produced lipase (61%), caseinase (57%), amylase (26%), gelatinase (16%),

cellulase (12%), chitinase (12%), and, for the first time in coral microbiota, keratinase (37%). Our study indicated that *M. alcicornis* represents the best source to identify more different enzyme types. However, the best isolate producer of different enzymes differed from coral to coral. This fact confirms the biotechnological and ecological importance of these microbial communities and the preservation of this ecosystem.

P SYM 8

Insights into termite symbioses from symbiont genomes and metabolomics

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In addition to their role as pests in urban environments, termites are keystone species and ecosystem engineers in numerous environments, particularly in arid areas. The ecological success of termites stems from their social behavior, and their unparalleled ability among animals to digest lignocellulose, the structural component of plant cells. The low nitrogen content of lignocellulose (~0.2% in wood) compared with N levels found in their tissues (~11%) means that termites must conserve this element. To do this they have developed intimate associations with microbes, which exist both in the hindgut, and - in the case of the giant Australian termite *Mastotermes darwiniensis* - in specialized cells of the 'fat body', an organ akin to the vertebrate liver. We have sequenced the genome of Blattabacterium cuenoti from the fat body cells of *M. darwiniensis*, as well as from cockroaches, which are close relatives of termites. Based on this data I will discuss the evolution and role of *B. cuenoti* in nitrogen recycling in its hosts. I will also present some insights into termite nitrogen metabolism from recent metabolic profiling of C13-cellulose-fed termites of a different species - *Hodotermopsis sjoestedti* - using 2D-NMR.

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P SYM 9

Entophytic bacteria isolated from the red alga *Laurencia glandulifera* as potential producers of bioactive compounds

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Marine bacteria produce a variety of secondary metabolites, due to antagonism that exists in their natural environment.
Our aim was to study the diversity of entophytic bacteria isolated from the red alga *Laurencia glandulifera* and to investigate their ability to produce secondary metabolites with biotechnological interest. The samples were collected from the body of the alga that originated from two different sites, within ten meters distance between each other. 129 entophytic bacteria were isolated using the serial dilution method and plating on agar media Nutrient Agar (NA) and Tryptone Glucose Agar (TGA). BOX-PCR analysis was performed for the differentiation of isolated bacteria using BOXA1R primers. The production of bioactive substances was studied using an antagonistic diffusion method on the agar media NA and TGA for 5 microbial indicators.
The isolated bacteria were differentiated into 95 strains and 57 of them were unique. Both sites exhibited 18% similarity in their microbial populations according Sorensen Similarity Coefficient. Four multiactive stains and the 8 other strains with the highest activity were selected (total 12) and identified by analyzing their 16S rDNA sequence. The multiactive strains appeared to have the major growth inhibition for two indicator strains, *Acinetobacter radioresistens* and *Micrococcus luteus.* The production of bioactive compounds was validated in the liquid culture's supernant and in extract solid cultures. These samples were separated in low and high molecular weight compounds with gel-filtration chromatography. The low molecular

weight fraction of *Bacillus cereus* 57A and *Bacillus cereus* 53A extracted from solid cultures, analyzed with reverse face high performance liquid chromatography to investigate the number of bioactive compounds.
Each one of the above bacteria produced 8 diver low molecular metabolites with potential biotechnological.

P SYM 10

Epi- and endophytic microbial communities of arctic and subarctic peatland mosses

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Northern peatlands constitute one of the largest natural sources of methane emission. Their inhabiting plant community is dominated by bryophytes of *Sphagnaceae* and Amblystegiaceae (brown mosses), that can be associated with microbial communities of the methane cycle. *Sphagnum* mosses prefer oligotrophic, acidic bogs and are known to be colonized by with methane-oxidizing bacteria. Brown mosses and their microbiome grow in mesotrophic, neutral wetlands and have been poorly studied, even though they reduce methane emissions from Arctic polygonal peatlands. Overall the role of methanogenic archaea associated with mosses is not clarified.
A variety of Amblystegiaceae and *Sphagnum* species from altogether 26 sites in Svalbard (Spitsbergen), Samoylov Island (Lena Delta, Siberia), Finnmark (Northern Norway) and temperate peatlands (Serrahn, Northern Germany) were collected in addition to reference samples (sedges, sediments). Separation of epiphytic and endophytic microorganisms in mosses was achieved by ultrasonic cleaning prior to surface sterilization. Epi- and endophytic bacterial and archaeal communities of mosses were analyzed by Tag-sequencing of 16S rRNA gene and functional genes (*mcrA*). Cell wall analysis (lignin, holocellulose, CEC) and C/N of mosses and sedges were carried out and environmental parameters (pH regime, DOC, organic acids) as well as temperature, CH_4 and O_2 gradients in pore waters were measured and used for statistical analysis.
The differences between epi- and endophytic microbial communities where little while geographic location and plant taxa shaped the structure both of bacterial and archaeal communities. Nevertheless brown mosses showed a more versatile microbial community than that associated with *Sphagnum* mosses. Microbial colonization also seems to be more pronounced in inundated sites.
For the first time we show the community structure of epi- and endophytic bacteria and archaea associated with *Sphagnum* and brown moss species and their environmental controls. It is known that environmental parameter such as pH and water level as well as biogeography shape microbial communities, which is also confirmed here.
Our data raise the question if moss-microbial-associations are rather influenced by moss species or environmental parameters.

P SYM 11

Effect of the plant flavonoid luteolin on *Ensifer meliloti*

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Ensifer meliloti (formely *Sinorhizobium meliloti*), a Gram-negative nitrogen-fixing proteobacterium distributed in soils both in free-living and symbiotic form, is considered a model bacterium for legume-rhizobium symbiosis[1]. It is well known to establish a specific symbiosis with leguminous plants by formation of specialized structures, known as root nodules. Within nodules *E. meliloti* reduces the atmospheric N_2 into NH_3. A successful symbiosis establishment depends on a complex signal exchange among the two partners. The early stage of signaling involves the release from plant roots of the flavonoid luteolin, which in turn induces the expression of nodulation (*nod*) genes via activation of the bacterial NodD transcriptional regulator[2].
To date, several extensive transcriptomic and proteomic analyses have contributed to characterize in detail the bacterial response to the luteolin perception at gene expression level[3,4]Despite the molecular information outlined, a global view on *E. meliloti* phenotypes affected by the flavonoid luteolin is still lacking.
An extensive phenotypic investigation of the symbiotic bacterium *E. meliloti* strain 3001 has been performed to elucidate the luteolin effects on the bacterial phenotypes using the high-throughput Phenotype MicroArray in combination with several dedicated assays.

Results revealed that the plant signal luteolin affects a wide spectrum of *E. meliloti* phenotypes. The strain displayed an enhanced resistance phenotype in presence of luteolin toward a broad set of chemicals including antibiotics, toxic ions, respiration inhibitors, membrane damages, DNA intercalants and other potential antimicrobial agents. Additionally, the presence of the luteolin significantly reduced the overall long-chain *N*-Acyl homoserine lactones production, as well as the *lag* phase depending on the starting cellular density, the motility and biofilm formation under nutrient-limited growth conditions. An effect on *E. meliloti* IAA production was also detected *in vitro* as a response to the luteolin.
Overall, these findings suggest that the plant signal luteolin plays a wide role in modifying rhizobial phenotypes, possibly in relation with plant root association and then symbiotic interaction.

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P SYM 12
Bacterial symbionts associated with a phloem-feeding heteropteran
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Bacterial symbionts are associated with diverse groups of phytophagous insects and confer fitness advantages to their respective insect host. These symbionts provide the host with nutrients, chemical signals, and/or protection against stress (*i.e.*, pathogens and xenobiotics). *Blissus insularis* (Hemiptera: Blissidae) is a destructive phloem-feeder of St. Augustinegrass and is notorious for its ability to develop resistance against a pyrethroid insecticide, bifenthrin. This insect has specialized midgut crypts harboring *β*-proteobacterial *Burkholderia* symbionts known for their ability to degrade xenobiotics (*i.e.*, insecticides). In this study, bifenthrin-resistant (R) and bifenthrin-susceptible (S) colonies of *B. insularis* were established. Using culture-dependent and -independent approaches, bacterial symbionts were examined to determine if the composition and density of *Burkholderia* inhabiting the R colony differed from that in the S colony. Genomic DNA extracted from the midgut crypts and the reproductive tracts of females were subjected to 16S rRNA PCR amplification and sequencing. Phylogenetic analyses of 16S sequences revealed a clonal association between *Burkholderia* and the crypts, whereas the reproductive tracts contained a bacterial community including *Burkholderia*, *γ*-proteobacteria, *α*-proteobacteria, and low titers of *Wolbachia*-like bacteria. The 16S sequences of crypt-associated bacteria obtained from R and S females did not form distinct groups in the phylogenetic tree, suggesting that the R and S phenotypes of *B. insularis* cannot be distinguished by the ribotype of their gut symbionts. However, qPCR reactions targeting 16S rRNA and *dnaA* genes showed that R harbored significantly higher gene copy numbers of *Burkholderia* in crypts than did S females. These findings suggest a potential link between the gut symbiont density and the host's resistance against insecticide. The *Burkholderia* isolated from R and S crypts cultured *in vitro* were further analyzed to determine the genomic features (using MLST, BOX-PCR), antibiotic susceptibility, plasmid profile, and pesticide-degrading capability. These results highlight the high affinity between *B. insularis* and symbiotic *Burkholderia*, providing a valuable model to structure our understanding of insect-bacterium symbiosis.

P SYM 13
Functional roles of bacteria in lichen-associated bacteria studied by comparative omics
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Lichens are one of their classic examples of symbioses as a self-stabilized and successful lifestyle on earth. In this symbiotic system, host-specific bacterial communities were identified in addition to the known roles of fungi as a morphological host and algae as primary producers. Their roles of bacteria remained largely elusive, apart from functions previously detected by us in the cultivable fraction. We aimed to explore the metabolic potentials of the microbiome using an omics approach with the lung lichen Lobaria pulmonaria as the model. Metagenomic and proteomic data were comparatively assessed and

visualized by Voronoi treemaps. The study was complemented with molecular, microscopic and physiological assays. More than 800 bacterial species were detected and they have a multifunctional potential in the symbiotic system, including: (i) nutrient supply, especially nitrogen, phosphorous and sulfur, (ii) resistance against biotic stress factors (that is, pathogen defense), (iii) resistance against abiotic factors, (iv) support of photosynthesis by provision of vitamin B12, (v) fungal and algal growth support by provision of hormones, (vi) detoxification of metabolites, and (vii) degradation of older parts of the lichen thallus. These results suggest the potential of associated bacteria to support growth and fitness of their lichen hosts. We present a comprehensive model of the symbiosis depicting the functional multi-player network of the participants. We argue that the strategy of functional diversification in lichens supports the longevity and persistence of lichens under extreme and fluctuating ecological conditions [1].
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P SYM 14

Use of an oligrotrophic culture medium enables captivation of diverse alphaprobacterial lineages from the marine sponge *Spongia* sp

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The use of traditional culture media to survey microbial diversity in natural ecosystems suffers from several constraints, of which incubation conditions and lack of precise nutritional factors required for growth rank among the most limiting. Alternative cultivation methods may help overcoming these hurdles, allowing the identification of microbial metabolic properties that cannot be discerned by molecular techniques alone. Here, we develop a procedure to isolate and select putatively novel bacterial symbionts from a marine sponge species of the genus *Spongia* (Dictyoceratida, Spongiidae). Our method involved spread plating of serially diluted, sponge-derived microbial cell suspensions onto an oligotrophic medium ("MG50", Marine Broth 1:50 solidified with gellan gum), designed to avoid overgrowth by copiotrophic bacteria and enable observation of bacterial colony development through longer incubation periods. In total, 706 bacterial colony-forming units (CFUs), retrieved from four different sponge specimens and averaging *c.* 10^6 CFUs per gram of sponge (fresh weight), were counted on MG50 after 8 weeks of incubation at 19º C. Whereas over 80% of the observed CFUs developed during the first two weeks, only 4% appeared by the end of the incubation period. Forty-five unalike CFU morphotypes were subjected to 16S rRNA gene sequencing for taxonomic identification and phylogenetic inference. We detected a high prevalence of alphaproteobacterial strains - at the expense of a much-reduced number of gammaproteobacterial isolates commonly recovered with full-strength media - spreading across diverse genera such as *Anderseniella*, *Erythrobacter*, *Sphingorhabdus*, *Leisingera*, *Loktanella*, *Tateyamaria* and *Thalassobius*. We conclude that MG50 is a preferable medium for the isolation of *Alphaproteobacteria* lineages usually not detected in regular cultivation surveys of the marine sponge microbiome. Future comparative genomics and metabolomics studies will shed light on their likely roles within this complex symbiotic consortium.

P SYM 15

The importance of biodiversity for the functional performance of microbial communities

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Introduction: Microbial communities perform functions that provide crucial services to ecosystems and human society: in wastewater they detoxify pollutants and consume environmental nutrients, thus mitigating the potentially deleterious effects of these chemicals on ecosystems and human health. However, the role of community composition and biodiversity for providing these ecosystem functions remains unclear.
Objectives: Our main objective is to address the following question: *When are community composition and biodiversity important for the provision of a particular ecosystem function and when are they not?*
We hypothesize that community composition and biodiversity are more important for rare ecosystem functions than for common ecosystem functions. While this hypothesis is intuitive, a recent meta-analysis contradicts it by demonstrating that

measuring the composition of microbial communities is not a good predictor for the performance of specific ecosystem functions.
Materials & Methods: To address this knowledge gap, we measured the kinetics of 35 independent wastewater treatment plant (WWTP) microbial communities to determine their functional performance. We will perform bioinformatic analysis of metagenomic and -transcriptomic data to identify rare functions and taxonomic and functional biodiversity.
Results & Conclusions: First results indicate a positive relationship between biodiversity and specific ecosystem functions among all tested WWTP communities. We observe that rate and yield are positively associated with each other among these microbial communities. When looking at 35 wastewater treatment plant communities, 42 out of 95 functions have a positive correlation between growth rate and yield, while the others have no or a negative correlation. This finding is consistent with biodiversity theory: high biodiversity has a positive impact on both growth rate and yield. While this theory applies on the individual level, we also observe a general tendency, correlating the mean yields and rates of 35 wastewater treatment plants. Wastewater treatment plants with low mean growth rates correlate with low mean yields and wastewater treatment plants with high mean growth rates with high mean yields (R= 0.83).
We are now investigating if the observed positive correlation can be explained by high taxonomic and functional biodiversity levels. We will then test if the specific functions, for which positive correlations can be observed, relate to the level of rarity or commonness.

P SYM 16
The ambivalent interaction between *Stenotrophomonas rhizophila* P69 and *Trichoderma* spp.
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Microbial cocktails have been proposed as a solution to overcome inconsistency, variability and non-reproducibility of biofertilization and biological control strategies under field conditions (Pal and McSpadden Gardener 2006; Berg 2009). However, before setting up multi-microbial applications it is important to assess the ecology of the interaction to determine compatibility of co-inoculants (Lutz *et al*. 2004).
In the present study, well known plant beneficial microorganisms such as *Stenotrophomonas rhizophila* P69 (Alavi *et al*. 2013), *Trichoderma velutinum* G1/8 and *T atroviride* P1 were used to gain new insights into the ecology of bacterial-fungal interaction (BFI) and into its potential synergistic effect on plant growth.
On tomato plants, the inoculation with *Trichoderma* spp. caused the significant increase of shoot biomass whereas the combination P69-G1/8 caused the significant increase of root biomass as well.
Growth and viability of *Trichoderma* strains were differentially affected by P69. On nutrient rich medium, all *Trichoderma* strains showed a slight transient growth reduction in co-cultivation with P69 or by exposition to VOCs emitted by P69. However, this effect was not observed under nutrient deficiency cultural conditions. In fact, P69 grown on poor medium did not affect G1/8 growth whereas it stimulated P1 growth. The effect of the interaction seems to be species-specific, and the availability of nutrients appears to play a key role in its modulation. Transcriptome data analysis of the interaction *S rhizophila* P69-*T atroviride* P1 will also be reported.
Bacterial-fungal interactions, although well known in nature, are underestimated, especially in relation to the potential effect that they might have toward third organisms which could be involved.

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P SYM 17

Hopanoids genes expression during *Methylobacterium mesophilicum* SR1.6/6 citrus interaction

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Methylobacterium mesophilicum SR1.6/6 is a pink pigmented bacterium able to use methanol as only carbon source, it was previously isolated endophyticly from citrus, promoting plant growth and inhibiting a citrus pathogens growth. However, the mechanisms or molecules involved in this interaction is not elucidated. In previous *M. mesophilicum* SR1.6/6 transcriptomic study we observed an up regulation in hopanoid biosynthesis genes during plant interaction. Theses molecules are lipids of cell membrane responsible for membrane stabilization, fluidity and permeability. It was previously reported to be a key component in stressful environment, in this way hopanoids might be related to plant-bacteria interaction. Moreover in *M. mesophilicum* genome, there is the complete pathway of hopanoid biosynthesis, including *hpn*A, *hpn*C, *hpn*D, *hpn*E, *hpn*F, *hnp*B, *hpn*L, *hpn*I, *hpn*J, *hpn*H, *hpn*N and *hpn*K genes. The present study aimed to evaluate the gene expression of hopanoid biosynthetic pathway in *M. mesophilicum* SR1.6/6 during *Citrus sinensis* (original host) interaction. *M. mesophilicum* SR1.6/6 cell suspension were inoculated in citrus axenic seedling and were kept at 28ºC for 5 days under agitation, three different treatments were evaluated: only SR1.6/6 strain (control); planktonic SR1.6/6 strain (influenced by plant exudates) and SR1.6/6 strain during citrus root interaction. RNA were obtained from all treatments, cDNA synthesis were performed and gene expression quantification were done by real-time qPCR. The results showed that overall the hopanoids genes were up-regulated in the presence of citrus plant, except for *hpn*E, *hpn*F and *hpn*H that were down regulated. Theses down regulated genes encodes dehydrosqualene reductase, squalene-hopane cyclase and hopanoid biosynthesis enzymes, respectively, all other genes responsible for side chain modifications in hopanoids are up regulated suggesting that hopanoids can be recycled and increased during plant interaction. Therefore, during *M. mesophilicum*-citrus interaction there are an increase in hopanoid production, that needs to be confirmed by lipids quantification. Finally, bacteria transcriptional profile can be associated with the maintenance and adaptation of bacteria during plant colonization.

P SYM 18

The efficiency of sugarcane colonization by arbuscular mycorrhizal fungi is modulated by distinct levels of soil microbial diversity

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Arbuscular mycorrhizal fungi (AMF) forms mutualistic association with many plant species. This interaction is often considered as a triple association, which involves the AMF, the plant host and the natural soil microbiota. The aim of this study was to evaluate the interaction of AMF species *Rhizophagus clarus* and *Dentiscutata heterogama* in sugarcane plants at vessels manipulated for distinct levels of soil microbial community diversity. Natural soil microbial communities were manipulated by a dilution-to-extinction approach (namely, 10^{-1}, 10^{-3}, 10^{-6} and 10^{-9}), and each AMF species was inoculated individually. We measured the rate of mycorrhizal colonization (%MC) and plant dry-weight (PDW) of samples at different times during the experiment (*i.e.*, 30, 60 and 120 days). For the treatment with *D. heterogama* species, all dilutions and sampling time presented highest rates of MC (30 to 50%) and similar values of PDW (30 days: 3.6 g to 5.3 g; 60 days: 7.6 g to 8,0 g and 120 days: 21,4 g to 20,5 g). For *R. clarus* species, the highest rate of %MC was observed at lower community dilution (10^{-1}), and the lowest rate %MC observed when the AMF was inoculated alone (difference ~50%), in particular after 60 and 120 days. At this sampling times, there was no relationship between the rate of %MC and the increase of DW, however, smaller colonization rates reflected in higher DW values (increasing value of ~30%). In conclusion, the inoculation of AMF species at distinct levels of soil microbial community diversity result in variable responses affecting the rate of colonization of AMF and consequently reflecting on the DW of sugarcane and the %MC rates.

P SYM 19

Exploring the hologenome concept in *Venerupis philippinarum* (Manila clam)

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The microbiota of some marine invertebrates has been extensively studied giving rise to the hologenome concept where species and their associated microbiota form the actual evolutionary entity. For bivalves study of positive interactions with the microbiota is still in its infancy. Recent studies suggest that members of their microbiota may form a microbial shield that could limit the settlement of pathogens.
We analysed the diversity of cultivable bacteria associated with *Venerupis phillipinarum* tissues (hemolymph, mantle and gills) collected in two sites, Aveiro (Portugal) and Arcachon (France), and characterized the bacterial isolates for protease production, biofilm formation and antimicrobial activity against aquaculture pathogens.
A total of 242 isolates: 75 from hemolymph, 79 from mantle and 88 from gills were obtained and identified by 16S rDNA sequence analysis. Families *Micrococacceae*, *Vibrionaceae*, *Pseudoalteromonadaceae*, *Bacillaceae* and *Pseudomonadaceae* were common to both sites but showed a different distribution by tissue. *Micrococacceae* were mostly associated to mantle, *Vibrionaceae* to gills and mantle and *Pseudoalteromonadaceae* to hemolymph. Overall *Vibrio* and *Pseudoalteromonas* were the most abundant genera. Even though microbiota composition differs between tissues and sites, Manila clams share some bacterial groups suggesting that these communities can be mostly *V. philippinarum* specific.
Sixty-six isolates (27.3%) were positive for protease production, most of them belonging to genus *Pseudoalteromonas* and *Vibrio*. Biofilm formation was positive for 21 isolates (8.7%) mostly members of the genus *Vibrio*. Regarding antimicrobial activity 32 isolates (13.2%), of which the genus *Arthrobacter* stands out, showed activity against at least one target bacteria. Of these, 24 showed activity against *Vibrio coralliilyticus* a coral pathogen that has been shown to elicit mortalities in fish and shellfish. Consistent with previous studies our data show that a low proportion of clam's microbiota could form a barrier community preventing bacterial infection. These results strengthen the hypothesis that bivalve microbiota can play relevant roles namely in the protection against invasive pathogens.

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P SYM 20

Bacterial cargo on symbiotic propagules of the lung lichen *Lobaria pulmonaria*

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Lichens are traditionally understood as an association of fungi with algae, but recent work revealed host-specific bacterial communities associated with this symbiosis as well. Little is known so far about the variation of the associated bacteria within the same lichen-fungal host species and across geographically separated populations, and how lichens recruit their bacteria. Vertical transmission of both fungal and algal partners in asexual propagules is a rather common reproduction mode found in many lichens. We were interested if bacteria are co-transmitted with these symbiotic propagules and if the asexual propagules could contribute to a geographical structure of lichen associated microbiomes. *Lobaria pulmonaria* was sampled from three localities in Eastern Austria and their associated bacterial communities were analysed by bar-coded pyrosequencing, network analysis and fluorescence *in situ* hybridization. Our results show that bacteria clearly colonize vegetative propagules of lichens. High-throughput sequencing analysis of the 16S rDNA fragment revealed a highly diverse bacterial community, not only on lichen thalli but also on the vegetative propagules. Propagules and entire thalli have a similar overall bacterial community structure at class level, except for the filamentous cyanobacteria *Nostocophycideae*, which were present only on thalli. All three sampling sites share a core fraction of the microbiome, with Alphaproteobacteria as the predominant taxon. Bacterial communities of lichen thalli from the same sampling site showed higher similarity than those of distant populations, which agrees with the hypothesis of a vertical short distance co-transmission of bacteria via symbiotic lichen propagules.

Reference:
Aschenbrenner IA, Cardinale M, Berg G, Grube M (2014) Microbial cargo: do bacteria on symbiotic propagules reinforce the microbiome of lichens? *Environ Microbiol*. 16(12):3743-52. doi: 10.1111/1462-2920.12658.

P SYM 21

Diversity of methanogens and co-occurrence with bacteria in feces in humans of different age and health status

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Hydrogenotrophic methanogens live in a synthrophic relationship with the human gut microbiota as the terminal part of the anaerobic food chain. *Methanobrevibacter smithii* of the *Methanobacteriales* is the prevailing archaeal species using hydrogen and carbone dioxide, or formate to produce methane. Recently, members of the novel order *Methanomassiliicoccales,* which use methanol and potentially methylamines as substrates, were isolated from feces. Whereas the intestinal microbiota-*M. smithii* association has been explored in-depth, little data exists on the prevalence, abundance, persistence and ecological relevance of methylotrophic methanogens in humans.
Therefore this study investigated the frequency and abundance of hydrogenotrophic and methylotrophic methanogens in healthy (n=13) and obese (n=13) children (8-14 years), and adults (n=17, 28-78 years) using quantitative PCR. From nine females, samples were obtained before and after giving birth. Bacterial groups linked to the abundance of methanogens were identified using a 16S rRNA gene amplicon dataset generated from the same samples.
94% of adults and 68% of children carried hydrogenotrophic methanogens whereas methylotrophic methanogens were recovered from 50% of the adults and one obese child. Overall relative abundance of methylotrophic methanogens in adults was lower than of hydrogenotrophic methanogens (0.09% vs. 0.54%). Hydrogenotrophic methanogens formed stable population in females before and after giving birth; in five females, methylotrophic methanogens were also repeatedly identified. Hydrogenotrophic methanogens co-occurred with bacterial genera associated with the trophic chain from carbohydrate degradation to hydrogen and formate formation. Relative abundance was inversely correlated to *Blautia* which encompasses acetogenic species.
In summary, hydrogenotrophic methanogens occurred more frequently and were more abundant than methylotrophic methanogens. Nevertheless, populations of methylotrophic methanogens were also temporarily persistent in some individuals. Negative correlation of methylotrophic methanogens with *Peptostreptococcaceae* and little characterized groups within the *Clostridiales* indicated possible interactions with the gut microbiota.

P SYM 22

Molecular "bridges" that build rumen microbial alliances

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Alliances between microbes and/or eukaryotes are implicated in processes of great importance to life on earth, from nitrogen fixation and mycorrhizal symbioses to assistance in digestion of food and biofilm formation. An important example of complex interactions between bacteria, archaea, fungi and protozoa can be found in the rumen microbial ecosystem within the fermentative forestomach of ruminants. These microbes interact to bring about the digestion of feed and play an essential role in the supply of energy to the host animal. The first step in the formation of these microbial partnerships is often adhesion to each other, mediated by surface-associated proteins. However, our understanding of the molecular mechanisms that drive these associations is hampered by the poor culturability of most rumen microbes and a lack of suitable genetic manipulation systems that might assist in unravelling these adherence mechanisms.
Using a culture-independent approach, based on metagenomics and phage display, we functionally identified the first adhesin from the symbiotic rumen methanogen *Methanobrevibacter ruminantium* M1, encoded by *mru_1499* (adhesin-like ORF). Affinity binding assays revealed that this "promiscuous" binding protein, Mru_1499, may facilitate adhesion to several rumen protozoal species and a xylanolitic rumen bacterium *Butyrivibrio proteoclasticus* B316. Identifying molecular mediators of adhesion between hydrogen-producers and methanogens, such as the adhesin Mru_1499, can significantly improve our knowledge about the mechanisms underlying these important alliances, and contribute more broadly to advances in microbial ecology of the rumen and biotechnological opportunities areas arising from such studies.

P SYM 23

Illumina sequencing unveils host-specific profiles and highly diversified microbial dark matter in marine sponge symbiont communities

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The question whether or not prokaryotic communities in marine sponges are host species-specific bears implications to the management of marine metabolic resources given the status of these holobionts as the most profuse source of biologically active compounds in the oceans. Here, we inspect microbiome community structures across four sympatric sponge hosts via high-throughput metagenomic DNA sequencing. Specimens of *Phorbas fictitius* (n=12), *Cliona viridis* (n=4), *Cliona celata* (n=7) and *Dysidea fragilis* (n=3) were collected at 19m depth off the Algarvian coast, South Portugal. Analysis of 630,000 Illumina HiSeq-generated 16S rRNA gene amplicons was performed with the mothur software package, delivering 13,238 prokaryotic operational taxonomic units (OTUs, singletons removed) across the data with each sponge specimen hosting averages of 1100 to 1600 prokaryotic OTUs. Nearly 65% of all the taxonomically assigned sequences were classified as *Proteobacteria*. Ordination analysis of OTUs revealed clearly host-specific symbiont communities and no evidence for higher similarities between microbiomes from closely related sponge hosts. Particularly, *C. viridis* exhibited a strikingly specialized cohort of so-far unclassified *Proteobacteria* species. The taxonomic richness of the sponge-associated "singletome", that is, the pool of rare OTUs containing one single sequence read, was exceedingly high, comprising 11,200 phylotypes several of which unclassifiable at the phylum or class levels, requiring future fine-tuned phylogenetic assessments. This study reveals species-specialized microbiomes across sympatric sponge hosts and a highly diversified, as-yet untapped, microbial dark matter associated with these animals, thus expanding our knowledge of the microbial genetic diversity encrypted in the sponge holobiont.

P SYM 24

The importance of intraspecific diversity of ectomycorrhizal fungi for regulating ecosystem functioning

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The relationship between biodiversity and ecosystem function is a hotly debated topic in ecology and yet little is known about the nature of the relationship in microbial communities. Furthermore, even less is known about the relative importance of within-species versus between-species diversity. The ectomycorrhizal (ECM) fungus *Laccaria bicolor* was used to test the hypothesis that genotypic diversity has a role in regulating ecosystem function when in association with a plant host. Microcosms containing pine tree seedlings colonized by a gradient of ECM genotypic diversity, from monoculture to a mixture of genotypes, were used to test for the ecosystem responses of plant and fungal productivity, soil CO_2 flux and nutrient loss in leachate. Microcosms with a gradient of ECM species were also assessed for comparison, and microcosms containing different complexities of nitrogen and phosphorus in the soil were used to investigate resource niche partitioning effects. Significant genotypic identity effects on plant and fungal productivity, and nutrient loss in leachate were found. There was a weak positive relationship between ECM root-tips per root length and genotypic richness, and nutrient loss generally deceased with increasing genotypic richness. Genotype mixtures outperformed the monocultures in half of the cases. Stable isotope and fungal culture experiments were utilised to further elucidate genotypic diversity selection effects, and suggested differences in fungal growth rates and nitrogen source uptake and utilisation. Genotypic effects were as significant as species effects, and results highlight that genotypic diversity is important for ecosystem function and selection and complementarity effects are both in operation.

P SYM 25

Comparative genomics reveals shared and specific *Vibrio* strains across multiple animal hosts

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Increasing evidence exists for host-specific symbiont communities in the marine realm. However, a few marine microbes emerge as typical generalists given their widespread occurrence in the open environment and across several host species. Among these, *Vibrio* bacteria are of particular concern given their recognized pathogenic potential. Here, we perform a fine-tuned genotypic characterization of *Vibrio* species cultivated from different marine animals. *Vibrio* strains were isolated from the marine sponges *Ircinia variabilis* and *Sarcotragus spinosulus*, the gorgonian coral *Eunicella gazella* and from gilthead seabream (*Sparus aurata*) larvae reared in aquaculture. Maximum Likelihood (ML) phylogenetic inference of 16S rRNA gene sequences obtained from 81 *Vibrio* isolates was performed with the ARB software, and entries sharing >99% ML nucleotide sequence relatedness were binned into operational taxonomic units (OTUs). In total, 16 OTUs were identified of which 4 OTUs comprised 41 isolates from gorgonians and sponges, with *Vibrio crassostrea* as the closest type strain; 5 OTUs embraced all 19 sequences originated from seabream larvae, sharing relatedness with *Vibrio chagasii*; and 1 OTU contained 8 sequences retrieved exclusively from gorgonians (*V. Ientus*/*tasmaniensis*). Representative strains from 8 OTUs were selected for full genome sequencing and found to display similar patterns of energy allocation across primary metabolic functions regardless of host origin. We depicted a generous investment in carbohydrate breakdown, highlighting exceptional nutrient scavenging capacities among the surveyed strains. Host-associated metabolic traits (e.g. chemotaxis, cell signaling, adhesion proteins) were assigned to 10-15% of the classifiable genes in the annotated genomes. Intriguingly, *Vibrio* spp. were found not to rank among the most dominant bacteria inhabiting gorgonian, sponge and seabream larvae according to cultivation-independent, 454-pyrosequencing microbiome profiling. Our results hint at both host-specialized and -generalist patterns of occurrence of rather low-abundant but likely opportunistic *Vibrio* species across disparate animal hosts. Ongoing, in-depth genome mining will shed light on adaptive traits likely conducive to generalist and specialized behaviors.

P SYM 26

Depicting the bacterial community associated with culturable cyanobacteria

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Cyanobacteria are essential primary producers in aquatic and terrestrial ecosystems, being the only prokaryotes capable of performing oxygenic photosynthesis. Members of this group play an important role in both carbon and nitrogen cycles. Most *cyanobacterial cells* are surrounded by mucilaginous matrix and exopolysacarides that can serve as nutritional source for heterotrophic bacteria, providing a long term and dynamic interaction. Continuous relationships over long periods may affect the evolution of the microorganisms involved. In this study, we investigated the composition and structure of bacterial communities associated with 36 cultivated cyanobacteria strains from eight different genera and isolated from several locations and environments. The independent-cultivation methods 16S rRNA PCR-DGGE technique (Denaturing Gradient Gel Eletrophorese) and massive sequencing of 16S rRNA gene were applied. Sequence analysis data showed that the main groups associated to cultured cyanobacteria were *Cytophagales, Flavobacteriales, Sphingobacteriales, Caulobacteriales, Rhizobiales, Rhodospirillales, Sphingomonadales, Burkholderiales, Pseudomonadales*. However, different abundances of these groups were found in each cultured cyanobacteria. The bacterial community composition was mainly determined by the cyanobacteria host genera (results from DGGE and sequence analysis), suggesting that the symbiotic communities associated to the cyanobacteria strains from distinct environments might follow the host dispersion. A more detailed analysis elected the rare community as the main responsible fraction of the community to this specificity. Therefore, we suggest that the bacterial community can fluctuate according to the 'host habitat', giving support for the inference on co-evolutionary studies.

P SYM 27

Transcriptomic analysis of differential gene expression of *Methylobacterium mesophilicum* SR1.6/6 during citrus interaction

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Methylobacterium mesophilicum SR1.6/6 is aerobic pink-pigmented facultatively methylotrophic bacteria (PPFMs), it has been described to host a range of plants as endophythes. This strain was isolated from healthy citrus plant and it has been the focus of several works in order to understand the interaction between host plants and SR1.6/6. Although many studies have been performed with *Methylobacterium* spp. during interaction with the host plant, much remains to elucidate and understand the mechanisms involved in the bacterium establishment and plant colonization. The aim of this work was to analyze the genes expression of SR1.6/6 after the colonization in *Citrus sinensis* using RNAseq. *M. mesophilicum* SR1.6/6 cell suspension were inoculated in citrus axenic seedling and were kept at 28ºC for 5 days under agitation, three different treatments were evaluated: only SR1.6/6 strain (control); planktonic SR1.6/6 strain (influenced by plant exudates) and SR1.6/6 strain during citrus root interaction. RNA of these treatments were obtained and carried to Solid sequencing platform. The results showed that in both interaction treatments many regulator and stress oxidative genes were up regulated in planktonic cells, while in cells adhered to plant root genes involved with biofilm formation and quorum sensing system were up regulated. The first step of any interaction between bacteria and plant, occurs involvement and initial recognition, that triggers a series of mechanisms mediated by molecular signals of the parties involved. Genes in response to oxidative stress may be responsible for protecting cells against a burst oxidative triggered by the plant, and these can be important in the growth and adaptation of the microorganism to the host environment and then some cells can enter into plant tissues and for this to happen some genes involved in surface adhesion can be key to establish this interaction. On the other hand regulators genes need to be more studied in order to understand its role during in bacteria-plant interaction.

P SYM 28

Characterization of the bacterial community associated to Pine Wilt Disease through culture-independent methods

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The Pine Wilt Disease (PWD) has huge impact on Eurasia pine forests. PWD results from the interaction between the causing agent, the pine wood nematode (PWN) *Bursaphelenchus xylophilus*, its insect-vector (*Monochamus* spp.) and the host tree (*Pinus* spp.). PWN associated bacteria were reported as toxins' producers causing PWD symptoms. Although less studied, bacterial community associated to the host tree and the insect-vector may also be important to PWD mechanism.
The aim of this study was to characterize and compare, through culture-independent methods, the bacterial community associated to PWD.
Six symptomatic *Pinus pinaster* trees were collected from each 3 affected regions both in Portugal mainland and in Madeira Island. Sawdust from each tree was used for nematodes extraction. Tree trunks were maintained in greenhouse for *Monochamus galloprovincialis* collection.
Total DNA was extracted from trees' sawdust, nematodes, and the tracheal system of the insects. *B. xylophilus* molecular detection was performed for all samples. V3 region of the 16S rRNA gene was analysed using denaturing gradient gel electrophoresis (DGGE) and barcoded pyrosequencing. DNA samples of each species were selected for pyrosequencing based on DGGE profiles.
While nematodes and insects are by far dominated by *Gammaproteobacteria* (82.9% and 78.7%, respectively), symptomatic trees have not only *Gammaproteobacteria* as major abundant class (36.8%) but also *Acidobacteria* (21.5%). *Pseudomonadaceae* and *Enterobacteriacea* are the predominant bacterial families in nematodes (24.0% and 35.0%, respectively) and insects (68.2% and 10.7%, respectively). Trees' samples are dominated by are *Xanthomonadaceae* (25.3%) and *Acidobacteriaceae* (22.3%). Nematodes' bacterial community is significantly different from its host tree ($p<0.05$).

Gammaproteobacteria is an abundant bacterial class in the three studied communities and might have a relevant role for PWD mechanism. Results suggest that *B. xylophilus* has a specific bacterial community that might be influenced by the insect-vector community.

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P SYM 29

Metatranscriptomics on termite and host associated symbionts to identification of biomass degrading enzymes

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Researches on second generation bioethanol production have been focused on seeking for enzymatic pre-treatment strategies for biomass delignification. Termites are insects that metabolize lignocellulose efficiently so, studies on how this mechanism works might be used as a source to find enzime candidates for biotechnology researches on biofuel production. Our goal is to identify and study gene candidates in the termites digestome, including host-associated symbionts, using metatranscriptome approach. Our focus is based on metatranscriptomics analyses of three termite species: *Conitermes cumulans*, *Velocitermes heteropterus* and *Heterotermes tenuis*. The insets were collected in Rio Claro-SP, Brazil, in 2010 and isolated by two cates: soldiers and workers. For each individuos The mRNA for each individuals (soldier and worker for three species) were extracted and sequenced by Illumina tecnologies generating ~60Gb data. Each species was assembled using Trinity assembler merging soldiers and workers sequences. The rRNA reads were used for taxonomic classification and the contigs were submitted to automatic annotation and differential expression anlysis in order to identify digestive enzymes in workers against soldiers. Proteobacteria, Firmicutes, Actinobacteria and bacteroidetes were the most representative *phyla*. Fibrobacteres, Fusobacteria and Deferribacteres were absent on H. tenuis. The transcriptome assembling resulted in ~96.000, ~96.0000 and ~212.000 contigs for *V. heteropterus*, *C. cumulans* and *H. tenuis,* respectively. We also identified digertive enzymes in symbionts and termite genomes. The differential expression analysis showed 416, 390 and 99 genes in *V. heteropterus*, *C. cumulans* and *H. tenuis*, respectivelly. These are preliminary results and future analysis include a better comprehension of comparative digestoma.

P SYM 30

Improving Truffles development. Rhizosphere and Mycorrhizosphere host different bacterial and fungal consortia.

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Advanced in metagenomic studies on host-associated microorganisms are beginning to greatly widen our knowledge of host interactions with mutualistic and commensal microorganisms. Bacteria and fungi host symbiosis have been suggested to influence the development of several truffle species in the different phase of their life cycle having a role in mycorrhizal symbiosis establishment.
The economic importance of the white truffle (Tuber magnatumPico) has encouraged attempts to its cultivation through artificial spore inoculations, but results are still poor due difficulties in obtained good mycorrhized seedlings.
Determine how the bacteria and fungi consortium combined with specific ecological condition influence the truffle development can greatly improve the chance of successful artificial cultivation.
To deeply characterize the structure of the microbial communities associated with the roots of truffle-inoculated we sequenced both the bacterial 16s and fungal ITS rRNA genes with the Illumina MiSeq system. We obtained approximately 12M bacterial and 10M fungal paired-end reads. Bioinformatic and statistical analyses were performed using USEARCH, QIIME and different R packages to describe the ecological patterns that occurs in this symbiosis. Results showed that complex bacterial and fungal communities of the ectomycorrhizosphere that differentiated from those of the surrounding soil and the rhizosphere. Main results were then presented and discussed in this contribution.

P SYM 31

First report of the occurrence of White Pox disease in a scleractin coral *Siderastrea stellata*

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Coral diseases are one of the most critical causes of reef degradation over the past decades. A greater part of these threats are related to anthropogenic impacts such as sewage releases, nutrient loading and eutrophication. White Pox (WP) disease, also known as acroporid serratosis, was originally, and only described in *Acropora palmata* on Caribbean reefs. WP disease is the first example of a human pathogen infecting a marine organism, and the pathogen, *Serratia marcescens*, is a common fecal enterobacteria described as cause of acquired infection at hospitals for the last decades and easily found in untreated sewage. This is the first report of the occurrence of White Pox disease in other coral than *Acropora palmata.* In this study, bacterial communities of healthy and White Pox infected coral tissues of *Siderastrea stellata* were analyzed from the Brazilian coast using fingerprint techniques and it confirmed the presence of *Serratia marcescens*. Our data shows an example of homeostasis breakdown due to the presence of microbial pathogen; that is because the profile of bacterial communities suffered a consistent impact on *S. stellata* colonies affected by white pox (MRPP tests; A= 0.20; p = 0.05). However, it was not observed (NS) any effect related to the place where colonies were sampled. Moreover, samples that were affected by White Pox (WP) disease were positive to *Serratia marcescens*, the etiological agent of WP disease. White pox-affected colonies had a significant prevalence of turf algae, but the relationship between algae e and White Pox disease is still unclear. Our data supportS the hypothesis that White Pox on *Siderastrea stellata* colonies is a bacterial disease caused by *Serratia marcescens*, but also demonstrates that this is not a specific pathogen of *Acropora palmata*.

P SYM 32

Alfalfa root symbionts under soil nutrient pressure: cooperation or competition?

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Understanding the role of root symbionts for plant nutrients uptake is of great importance in agro-ecosystems as they can directly impact the availability of nutrients in soil as well as crop plant growth and productivity. In a previous study, microbial eukaryotic community was observed to significantly change due to long term farmyard manure (FYM) fertilization, while mineral (NPK)fertilization did not appeared to impact their diversity and community composition (**1**). In order to describe more deeply the process governing root symbionts assemblages and their reaction to variable nutrient contents, Rhizobiales and Glomeromycota (AMF) from the rhizosphere of *Medicago sativa* were assessed by high-throughput pyrosequencing of the bacterial and fungal SSU rDNA from the Bad Lauchstädt long term static fertilization experiment (Germany). The detection of AMF was nearly impossible in soil fertilized with both FYM and NPK suggesting a loss of mycorrhization due to high nutrient availability for the plant. On the contrary, rhizobiales OTUs relative abundance positively correlated with C/N ratio and ergosterol (one way ANOVA, $p < 0.01$) while no significant correlation were observed with the total organic carbon and total nitrogen content of the rhizosphere soil. A network analysis will further be applied to test the correlation between AMF and Rhizobiales OTUs to assess possible plant selection strategies towards root symbionts along the fertilization gradient.

Reference:

1. Lentendu G, Wubet T, Chatzinotas A et al. (2014) Effects of long-term differential fertilization on eukaryotic microbial communities in an arable soil: a multiple barcoding approach. Molecular Ecology, 23, 3341-3355.

P SYM 33

Random mutagenesis of endophytic *Burkholderia seminalis* suggest that the gene cluster related to capsule synthesis is associated to inhibition of plant pathogens

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Endophytic strains of *B. seminalis* showed the ability to control the orchid necrosis caused by *B. gladioli* in *Oncidium* Aloha Iwanaga and *Oncidium* Sharry Baby in Brazilian production as well as to inhibit phytopathogenic fungi such as *Ceratocystis* spp., *Fusarium* spp. and *Colletotrichum* spp. Members of the *Burkholderia* genus are well known for their potential as human pathogens, their abilities to fix nitrogen and solubilize phosphate or their ability to control plant pathogen. However, the genetic determinants necessary for the latter function are not well described. To this end, we used random mutagenesis to generate a mutant library, containing 3,840 transposon mutants of the *B. seminalis* strain TC3.4.2R3. The library screening allowed us to identify 2 essential loci to biological control of orchid necrosis and fungi inhibition. The first locus was identified in the *wcb* cluster that is related to synthesis of the cell capsule, a key determinant in bacterial-host interactions in other systems; the second locus was identified in the O-antigen cluster that is related to induce systemic plant resistance. In the *wcb* and the O-antigen cluster Glycosyltransferase and Methyltransferase genes, respectively, were identified, suggesting that these genes could be related not only to the capsule synthesis, but also to molecules related to microbial interaction into the environment, such as plant interior. Genetic screens coupled with genome studies will yield a number of candidates loci that contribute to understanding the mechanisms necessary for *B. seminalis* strain TC3.4.2R3 to establish inside the host plant and control plant pathogens.

P SYM 34

Molecular and ecological interactions between plant pathogen and biocontrol strains of Burkholderia

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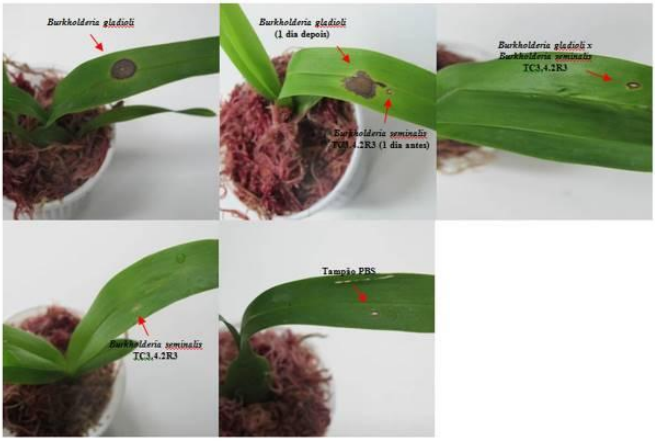
The *Burkholderia* genus consists of Gram-negative bacteria, which have a high physiological and genetical diversity. The bacteria belonging to this genus are the subject of studies and had been widely used for a variety of biotechnological applications as biocontrol agents, plant growth promoters, bioremediation / biodegradation and also as a producer of molecules of interest in medicine, agriculture and industry. It was investigated the interaction between *Burkholderia seminalis* TC3.4.2R3 and *Burkholderia gladioli,* inside the plant of *Oncidium* Alowa Iwanaga. The *B. gladioli* is a pathogen for several hosts that causes injuries in plant tissues like a soft rot diseases. A mutant library of *B. seminalis* generated by the random insertional of transposon Tn5 was analyzed, in wich was observed that the mutants in glutamate synthase, fatty acid desaturase and intracellular depolymerase beta polyhydroxyalkanoate genes, had reduced their ability to colonize in *Oncidium* and the controlling the symptoms of *B. gladioli*. Such genes may be involved in detoxification mechanisms and adaptation to environmental stresses, and may confer advantage in colonization and competition with other microorganisms. For mutants of glycosyl transferase, membrane transporters and the patatin, this reduction in colonization rate was not observed, which may be the subject of study for other biological control mechanisms of soft rot. It was observed that plants inoculated with bacterial isolates had reduced release of hydrogen peroxide in three hours, compared to the control, indicating that these bacteria, both *B. seminalis* and *B. gladioli* incorporating a detoxification system of reactive species oxygen, which allows the successful colonization of the host plant. The results have directed the hypothesis that the control of *B. seminalis* it is a direct control over *B. gladioli*, and not the induction of plant defense responses is the central mechanism of this control. Further studies of interaction between *B. gladioli* and *B. seminalis* inside de *Oncidium* Alowa Iwanaga tissues are being conducted by the analyses of the transcriptome of co-inoculetd plants in order to evaluate which genes are differentially expressed during this interaction.

Figure 1



P SYM 35

Functionality of honeybee (*Apis mellifera*) LAB member symbionts

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Current decrease of both wild and domestic pollinator populations has a negative impact on global food supply, raising new set of challenges. Protective measures aiming the maintenance of healthy insect habitats should be based on the fundamental knowledge of biotic and abiotic factors shaping sophisticated insect ecosystems. Among those biotic factors role of microbial symbionts remains to be explored and studied in detail.
Lactic Acid Bacteria (LAB) symbionts of honeybees are supposed to play key role in colony functioning: acting as an immune boosters, restricting pathogen invasion, aiding nutrient uptake and participating in beebread formation. Pollen is protein rich food of honeybees. The lactic fermentations were suggested to be protective factors beebread against deleterious microbial spoilages. Insufficient data has been provided in scientific literature on the microbial ecosystem of the beebread. Presented study aimed: to describe a role of *Apis mellifera* colony microbial dwellers by identification of the functions of indigenous LAB in bee life cycle.
Five samples of beebread were taken from one healthy honeybee colony, fungal strains were isolated, identified and used in experiments, where pollen fermentation was carried out in laboratory conditions using beebread LAB isolates. SDS PAGE, Starch Agar Plate & Well Diffusion methods were used to determine proteolytic, amylolytic, antibacterial and antifungal activities of those microroganisms as key features predetermining their symbiotic nature.
In total, 15 fungal strains were identified. Only 3 among all isolates: *Candida sp*., *Zygosaccharomyces rouxii* and one fungus *Aspergillus niger* were able to replicate on the pollen substrate. Growth of those fungal strains was inhibited in presence of LAB strains: *Lactobacillus kunkeei, Fructobacillus tropaeoli* and *Fructobacillus fructosus*. No proteolytic, amylolytic or antibacterial activities was detected.
Indigenous LAB strains display niche specific character taking part in beebread formation as effective fermentators and limiting the growth of fungal flora, thus preventing the spoilage. Fungus *Aspergillus niger* against which the above-cited LAB strains are active, is causative agent of honeybee larvae disease - stonebrood, which causes considerable damage in industrial beekeeping.

P SYM 37

Contribution of gut symbionts to the host physiology of a wood-boring beetle

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Psacothea hilaris hilaris is a xylophagous beetle belonging to the Cerambycidae family. Its larvae grow inside mulberry tree and fig trunks, reaching the length of up to three centimetres and damaging the plants to death. In Japan, this is a serious pest for sericulture, while its recent establishment in northern Italy could threaten the fig cultivation in the Mediterranean basin.
In this study, we characterized the gut bacterial communities associated to wild and laboratory-reared *P. h. hilaris* larvae by molecular and culture-dependant methods. PCR-Denaturant Gradient Gel Electrophoresis (DGGE) was applied to assess the significant effect of the diet and the gut tract in modifying the taxonomical composition of the gut bacterial community. Field-caught larvae and larvae reared on diet in presence of antibiotics and preservative showed richer communities than larvae reared on artificial diet, not exposed to antibiotics and/or preservatives; in this last case the larvae were dominated by *Enterococcus* and *Leuconostoc* sequences. This probably was due to the overgrowth of these microbes on the diets where antibiotics and/or preservatives were removed. PCR-DGGE analysis showed a significant difference in the bacterial community composition between the midgut and the hindgut, probably correlated to the different physiological conditions. By using a collection of bacterial isolates from the guts of wild larvae we evaluated the possible contribution of the isolates to the host physiology (in terms of contribution to carbon or nitrogen uptake) through *in vitro* tests. Results suggested that symbiont isolates can contribute to the cellulose digestion or exploit the by-products of the degradation of cell wall compounds and help their host to absorb nitrogen converting waste molecules (uric acid and urea) or proteins to ammonia and smaller peptides, or even fixing atmospheric nitrogen.
From our observations, the cultivable gut bacterial community of *P. h. hilaris* appears to include many different commensals, which are suitable to exploit the food sources in the gut and to give a contribution to the host metabolism.

P SYM 38

Bacterial symbiosis in dual-breathing animals living in mangrove ecosystem

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Tropical mangroves are coastal habitats with harsh environmental conditions predominantly due to tidal cycles that determine sharp gradients of salinity, oxygen and nutrient availability and of toxic compounds like plant-produced tannins and polyphenols and biogenic sulfide. The sediments are deeply perturbed by crabs that contribute to shape the carrying capacity the system. To exploit the sediments and the aerial plant root system several crab species evolved a capability to breathe in air and water allowing the exploitation of a larger niche range. Such a capacity determined a major functional evolution of the gills toward a lung-based function supporting a bimodal breathing and the catabolite excretion. We hypothesize that bacterial symbiosis is a major force shaping such a unique adaptation of the crab gills to cope with the mangrove ecosystem challenges. We focused on two mangrove key stone crab species, the ocypodid *Uca urvillei* and the sesarmid *Perisesarma guttatum*. Crabs were sampled in a large latitudinal range at their southernmost, northernmost and equatorial distribution sites respectively in the South African and Kenyan Indian Ocean coasts and the Red Sea Saudi Arabian coast. PCR-denaturing gradient gel electrophoresis, Illumina sequencing and optical, fluorescence *in-situ* hybridization and electron microscopy techniques, detected the constant presence along the latitudinal transect of a core bacterial community specific of each of the two species. The two communities were dominated by uncultured actinobacteria related to the genus *Ilumatobacter* at all the latitudes, while the other relevant group of the Rhodobacteriacee was represented by different OTUs in the three latitudes. These bacteria showed a specific location on the gill surfaces with a uniform colonization pattern in between the regularly spaced lamellae. The possible function of these bacteria is discussed in the light of their taxonomic identity and the localization on the gill surfaces.

P SYM 39

Habitat visualization and genomic analysis of "*Candidatus* Pantoea carbekii", the crypt-dwelling primary symbiont of the brown marmorated stink bug

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Phytophagous pentatomid insects negatively impact agricultural productivity and the brown marmorated stink bug (*Halyomorpha halys)* is an emerging invasive pest responsible for damage to many fruit crops in North America. Phytophagous stink bugs, including *H. halys,* harbor gammaproteobacterial symbionts that likely contribute to host development, and characterization of symbiont transmission/acquisition and their contribution to host fitness may offer alternative strategies for managing pest species. "Candidatus Pantoea carbekii" is the primary occupant of gastric caeca lumina flanking the distal midgut of *H. halys* insects and it is acquired each generation when nymphs feed on maternal secretions following hatching.

Question: Where is "Ca. P. carbekii" located on egg surfaces prior to nymph consumption and what of it genetic repertoire may contribute to host diet and development?

Methods: The complete "Ca. P. carbekii" genome was assembled from short paired-end reads and annotated using publically-available protein and nucleic acid motif databases with in-house Perl scripts. "Ca. P. carbekii" cells on egg surfaces and in host tissues were imaged using fluorescence in situ and, separately, electron microscopy methods.

Results: The "Ca. P. carbekii" chromosome is one-fourth (1.2 Mb) that of free-living congenerics and retains genes encoding many functions that are potentially host-supportive. Additionally, the genome includes multiple plasmids that encode components for thiamine biosynthesis and nitrogen recycling. Observed gene content in "Ca. P. carbekii" reflects patterns of gene loss/retention typical of intracellular mutualists of plant-feeding insects, suggesting a functional convergence around share host trophic strategies. Electron and fluorescence in situ microscopic imaging of *H. halys* egg surfaces revealed that maternal extrachorion secretions were populated with "Ca. P. carbekii" cells and it is these secretions that are consumed by nymphs and provide the initial inoculum of "Ca. P. carbekii".

Conclusions: The reported findings detail a transgenerational mode of symbiont transmission distinct from that observed for intracellular insect mutualists and illustrates the potential additive functions contributed by the bacterial symbiont to this important agricultural pest.

Oral presentations

O FOO 1

How mutualism evolves: Experimental Microbiome Evolution in gnotobiotic flies

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Metazoans harbour considerable numbers of commensal microorganisms in the gut, named microbiota, that contribute to many aspects of normal host physiology. Scientific research has provided invaluable contributions to our understanding of the relationship between host and its microbiota; however, the molecular mechanisms through which the microbiota exerts its beneficial influence are still largely undefined. The meta'omics revolution is promoting an explosion of interest in how the gut bacterial community impacts physiology and propensity to disease. In addition, gnotobiotic model organisms provide unique opportunities to study host-microbiota interplay with a level of experimental control that is not achievable in human studies. Our present project couples these two approaches to understand the bacterial genetic pathways mediating host benefits. To this end, we are applying *in vivo* experimental evolution to mutualistic bacteria, using gnotobiotic animal models. The work investigates the association between the host model *Drosophila melanogaster* and one of its most abundant commensal bacteria, *Lactobacillus plantarum*, which was demonstrated to be beneficial to its host physiology by promoting juvenile growth and maturation. We started experimental evolution (EE) approaches by mono-associating germ-free *Drosophila* embryos with a poorly growth-promoting *L. plantarum* strain. The EE setup has been propagated for more than 20 fly generations and it is still on-going. The selection pressure that is applied favours the most beneficial strains to adapt to the host's gut, a mechanism that will lead to the enrichment of those bacterial genes implied in host growth promotion. Indeed, after 250 bacterial generations the evolved bacteria improved their host growth promotion effect, both by increasing larval size up to 25% compared to the ancestor and also by reducing the fly developmental time up to 4 days. I will report the results about the evolution of the host/bacteria phenotype together with the genotype results obtained through comparative genome analysis between the evolved strains and the ancestor. Such data will shed light onto the genes that went through a higher selective pressure by accumulating mutations, which will be the candidate markers for the growth promotion effect driven by the microbiota.

O FOO 2

A mechanistic view of methanogenic modulation in the rumen

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Introduction. Methane emission from ruminant livestock constitutes a considerable portion from the global greenhouse gas emission. This emission is the outcome of methanogenesis activity of the methanogenic archaea that reside in the rumen. The effect of diet on the composition of rumen methanogens is ambiguous. Furthermore, the mechanism that govern these changes is not clear. Hence, in the present study, we aimed to characterize the overall archaeal communities' composition under two extreme diets and offer a possible mechanism that controls these differences.
Material and methods. Both experimental diets consisted of the same dietary fiber and varied only at its ratio. These diets were fed to 5 Holstein Friesian cannulated cows in a 69 days experiment which was divided into two feeding periods. On the first period, day 1-39, the cows were fed 95% fiber diet (high fiber diet). On the second period, starting from day 43 until day 69, 3 experimental cows were fed 30% fiber diet (low fiber diet) and 2 cows remained on 95% fiber diet as a control. In each feeding period, rumen content from each of the 5 cows was sampled every 4-7 days. The DNA from those samples was extracted and administrated to illumina pyrosequencing of the archaeal 16S rRNA gene. In order to determine the kinetics of the change within the different methanogenic orders, Quantitative RT-PCR was employed on all rumen samples taken in this experiment. Furthermore, GC and GC/MS were used to characterize the metabolome properties of the diets and finally *in silco* and genomic analyses were conducted in order to obtain a possible mechanism that controls the methanogenic communities' composition.
Results and conclusions. Our findings reveal that while the overall archaea abundance did not change with the diet, substantial shifts in taxonomic composition of the methanogenic communities were documented as revealed by pyrosequencing and qRT-PCR results. Some archaeal operational taxonomic units (OTUs) were highly diet specific and could be found in all samples from a specific diet rather than the other. These novel findings may have important future implications both at the rumen microbial ecology and at the environmental greenhouse gas perspectives.

O FOOD 1

Indonesian Tempeh as Functional Food:
Role of Bacterial Community as revealed by
Conventional Microbiology and Next Generation Sequencing Analysis

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Tempeh commonly refers to soy-based fermented food originated from Indonesia that has been widely recognized as an excellent source of both vegetable protein and vitamin B12. Although strains of *Rhizopus oligosporus* are known as the key players in tempeh production, Indonesian tempeh contains a garden variety of molds, yeasts, and bacteria up to 10^9-10^{10} cells per gram of fresh tempeh. Some of these microorganisms play important roles in determining tempeh quality such as the development of flavor, texture, and production of vitamin B12. On the other hand, the presence of these bacteria could contribute to a highly varied tempeh quality.
Tempeh samples from two different production systems were selected for immunomodulatory study as well as bacterial metagenome analysis employing Next Generation Sequencing (NGS).
Our results indicated that the dominant bacteria present in all tempeh samples were Firmicutes from the genus *Lactobacillus* that represents approximately 50% of the total frequency of 16S rRNA reads. Another dominant group (approximately 48%) was either *Acetobacter*, or *Klebsiella* depended on the tempeh samples. Further bacterial genome analysis, employing *Enterobacterial Repetitive Intragenic Palindrome* Polymerase Chain Reaction (ERIC-PCR), indicated that all of tempeh isolates were genetically different from those of *K. pneumoniae* pathogenic to human. Most of citrate-negative coliform isolates were identified as *Escherichia coli*. However, not all tempeh samples carried *E. coli* and all of the *E. coli* isolates derived from tempeh were genetically different from *E. coli* strains pathogenic to human.
Compared to non-fermented soybean, tempeh could significantly increase the expression of IgA mRNA in rats. Cooked tempeh elevated IgA transcripts similar to that of fresh tempeh, and therefore could be considered as a unique source of paraprobiotics.

O FOOD 2

Comparative metagenomics from oral microbiomes of hunter-gatherer and farmer populations from the Philippines

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The oral microbiome, the complex ecosystem of microbes inhabiting the human mouth, is composed by hundreds of bacterial species and plays a key role in human health, as the balance in the microbial community structure can confer protection against invasion or proliferation of pathogens. The effects of human sociocultural evolution affecting diet and lifestyle have been associated with the upsurge of chronic oral disease, which is endemic in industrialized societies but seems less common in both ancient and extant hunter-gatherers. While much is known about individual species associated with pathogenesis, the study of co-evolutionary dynamics between host and commensal microbiota remains a largely novel field of investigation.
Here we investigated the oral microbial diversity of three pairs of populations of hunter-gatherers (HG) and agricultural groups from the Philippines that live in close proximity and share similar natural resources. We applied deep shotgun sequencing of saliva-derived DNA, generating both microbiomes and associated human genomes at high coverage.
Diversity of the salivary microbiome was reconstructed from a set of 33 conserved marker genes extracted from the bulk metagenomic DNA and was explored using an explicit phylogenetic framework and multivariate statistical analyses.
This approach revealed unprecedented patterns of variation in community composition occurring at both shallow and deep phylogenetic scales. Both phylogenetic diversity and community composition discriminate HG groups from neighboring farmers. These differences became even more evident once we included microbiome samples representing a 'western-diet' lifestyle. We recover a gradient of microbial diversity with Western and HG diet occupying the low and high ends of the spectrum, respectively. We could identify a set of bacterial lineages whose variation in abundances correlates with this gradient, including *Haemophilus influenzae*, *Neisseria meningiditis*, *Veillonella parvula* and *Prevotella histicola,* suggesting differences in fitness of these taxa across the lifestyle gradient and/or preferred interactions with host factors.

O FOOD 3

Gut microbiota diversity of omnivore, vegetarian and vegan healthy subjects by culture dependent and rRNA DGGE profiling

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In the last decades several studies report the importance of improving the knowledge of how lifestyle factors such as diet, age or geographic site influence the change in gut microbiota. In particular, diet habits appear to be an important factor affecting the gut microbiota. Three main dietary habits are worldwide diffused: omnivore, ovo-lacto-vegetarian and vegan. The aim of this study was to assess the fecal microbiota of 153 volunteers (51 per category) recruited from North to South Italy between 30-50 years of age and with a male:female ratio approximately 1:1. The fecal microbiota was assessed at species level by RNA-based-DGGE and by using conventional enumeration on selective agar plates. The similarity matrixes obtained from dendrograms analysis of the RNA-DGGE fingerprints were used to build Projection on Latent Structures - Discriminant Analysis (PLS-DA). Concerning the dietary habits it was possible to observe a gradient of samples driving a certain degree of separation of omnivore from non-omnivore subjects. When the samples were grouped based on the dietary habits, the PLS-DA models showed a trend of differentiation based on geographical origin of the samples. It was observed that the fecal microbiota of ovo-lacto-vegetarian and vegan volunteers showed significantly lower counts of *Bacteroides fragilis* and LAB group. The food consumed can have an impact on fecal microbiota, long-term diets associated with low levels of protein and animal fat intake decrease the levels of the genera *Bacteroides* and the absence in vegan diet of food such as yogurt and cheese, decrease the gut LAB populations. All together, these findings confirm that the food consumed, more that the dietary habits, and geographical origin can have an impact on fecal microbiota. This work can be the basis for further research regarding the identification of biological, molecular and metabolic markers specific to the type of diet.

Poster presentations

P FOOD 1

New insights into the ecology of supralittoral sediments detritivors from the analysis of gut microbiota of talitrid amphipods

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Talitrid amphipods (sandhoppers and beach fleas) are supralittoral zone dwellers and obtain most of their food from stranded materials, which include detrital marine angiosperms and macroalgae, as well as occasional death animals. Here, we report the characterization of gut microbiota of Talitrus saltator, Talorchestia ugolinii, Sardorchestia pelecaniformis, Orchestia montagui, collected in Sardinia (Italy). Microbiota were analyzed by metabarcoding analysis on amplified 16SrRNAV4 region and by quantification of family 48 glycosyl hydrolases genes, which are involved in cellulose degradation. Obtained results indicated the presence of a complex bacterial flora, including several members of Verrucomicrobia in four out of the five species. Moreover, different gut microbiota taxonomic assemblages among the selected talitrid species were found. In particular, O. montagui (which lives in close contact with Posidonia banquettes) gut microbiota was found to be the mostdifferent with respect to those of the other talitrids, being more abundant in members of Firmicutes, Planctomycetes and Actinobacteria, and containing the highest level of family 48 glycosyl hydrolases genes. We conclude that talitrid amphipods harbor a complex gut microbiota which may be related to the habitat the different species colonize

P FOOD 2

The microbiota from choanae of selected free-living birds species

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Birds (Aves) are very successful group of vertebrates that inhabit diverse ecological niches around the world. Because of their vast numbers they have a big impact on environment. The studies that were already conducted on the microbiota of birds were directed towards particular pathogenic bacteria that infect the bird and are the cause of zoonotic diseases. Our study was focused on the composition of the bacterial microbiota that inhabit choanae. This knowledge is important as some free-living bird species represent a known way to transmit zoonotic diseases, but also for conservation and reintroduction efforts. We tried to determine choanal microbial diversity of selected free-living bird species. Because only a few researches focus on this specific topic, we also tried to determine presence of any bacterial pathogens. In 2014 we took samples from 44 birds, belonging to 17 different species. We determined bird sex and specie along with measuring of wing length and bird weight. The samples were taken with cotton swab, transferred to laboratory and immediately inoculated on plates with nutrient agar to suit nutritional demands of a big variety of bacteria. From a diverse collection of colonies 36 different colonies were isolated based on phenotypic differences and analysed further. Later we used commercial kits to isolate DNA from these isolates and identified them using 16S rRNA gene sequencing. We determined 31 different species including 3 potentially novel species (*Aeromicrobium* sp., *Agromyces* sp. and *Chryseobacterium* sp.). Most of them belong to phylum Actinobacteria (45.7%), other to phylum Proteobacteria (34.3%), Firmicutes (17.1%) and Bacteroidetes (2.9%). We concluded that choanae is an environmental reservoir for different bacteria, among them also some rare species, such as *Frigoribacterium faeni* and *Okibacterium fritillariae*, which needs further investigation.

P FOOD 3

"DIFFERENCES OF BACTERIAL COMMUNITY COMPOSITION BETWEEN CAECUM AND COLON OF RATS AFTER LONG TERM DIETARY INTERVENTION"

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Background: Host-microbe interactions are now considered essential for maintaining host health. Our understanding of forces shaping the gut microbiota is still limited and controversial, and most of the studies of the gut microbiota use the microbiota from faeces as a proxy for the intestinal tract populations.
Objective: Compare the microbial communities in caecum and colon of rats under three experimental diets.
Methods: Forty-five, 21-day old female (Wistar) rats, were fed one of three different diets: 5% fibre, 26% fibre (insoluble cellulose) and a diet consisting of 50% of the 5 % fibre diet and 50% cooked red kidney beans (rich in soluble fibre). After 14 weeks on the experimental diets the caecal contents and faeces of each animal were sampled. The microbiota was characterised by means of 16s (V4 region) amplicon sequencing using the Ion Torrent platform. Mothur was used to process the sequencing reads and composition of the microbiota was determined by referencing the reads to the SILVA bacteria database. Non-metric multi-dimensional scaling (nMDS), PERMANOVA, and similarity percentage (SIMPER) tests were performed.
Results: Two-way PERMANOVA showed an effect of diet ($p<0.001$) and sample location (caecum vs faeces)($p< 0.001$), but no interaction effect ($p=0.306$). In animals on the low and high non-fermentable diets, community differences between the caecum and colon were largely due changes in the relative abundance of the Actinomycetales, Peptostreptococcaceae, and Veillonellaceae, while in animals on the bean diet the differences were mainly due to Actinomycetales, Bacillaceae, and Lactobacillaceaea.
Conclusions: Findings support evidence for gut ecosystem manipulation. Dietary treatment promotes growth of specific members of the gut microbiota with significant changes at family level depending of the gut region.

P FOOD 4

***Escherichia coli* in poultry meat: prevalence, abundance and phylogenetic profiles.**

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Retail poultry meat products are frequently contaminated with *E. coli* and therefore people may acquire *E. coli* through ingestion of poultry meat products. *E. coli* is a common cause of bacterial infection in humans. Poultry meat is also thought to harbour the most "human-like" strains of *E. coli*, and may be a potential source of enteropathogenic and ExPEC (extraintestinal pathogenic *E. coli*) strains.
To provide insights into the attributes of *E. coli* isolated from poultry meat products sold in Australia with special attention to their prevalence, abundance and phylogenetic profiles.
Poultry meat samples were collected during 3 seasons from 16 shops. These shops represented 3 major supermarket chains that were co-located in the 4 major town centres of Canberra, Australia. Additionally, an independent butcher was sampled in each town centre. *E. coli* was isolated following enrichment using lauryl sulfate broth or Luria broth plus vancomycin, and also by antibiotic selection. All isolates were assigned to a phylogenetic group using Clermont quadruplex PCR and unique strains in a sample were identified by DNA fingerprinting using repetitive element palindromic PCR (REP-PCR).
E. coli was detected in 77.5% of 306 meat samples. Phylogroup A strains were most common among the 3415 *E. coli* isolates, with phylogroup B2 strains being the least common. The B2 strains are potential human ExPEC strains while A strains are typically over-represented in live chickens.
Identical strains were observed from different meat types, retailers, and geographic locations. The number of strains per sample varied with independent butchers' vs major retailers, meat type and mode of production (conventional, free range or organic). Poultry meats from independent butchers were less likely to have *E. coli* than the major retailers. Gizzards of whole meat had higher incidence of *E. coli* than mince, breast or wings. Products from organically reared or conventionally reared chickens were more likely to have *E. coli* than products from free range chickens. *E. coli* detection was significantly high during summer than autumn and intermediate in winter.
The results of this study provide further evidence that the food we consume is a significant source of *E. coli*. The results also suggest that some post processing contamination of poultry products may be occurring.

P FOOD 5

Effects of diet and predation on fish gut microbiota

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Gastrointestinal tract (gut) and their colonized microbiota are in a symbiotic relationship. Gut offers suitable ecological niches for the microbiota[1], which in turn provides essential functioning to the host[2], e.g. digest food, protection against pathogens, etc. Many studies found this relationship to be affected by diet, which is the most frequently studied one[3]. Meanwhile, besides diet, many other factors could also have strong effects on gut microbiota, for example, stress. Stress is quite common not only for humans but also for wild animals, e.g. fish facing predation. Stress could have impact on animal behavior, such as foraging behavior under predation risk. Stress-induced changes in nervous system and immune function can also lead to the changes on gut secretion and motility, which will influence the stability of the microbes living in gut[4]. In this study, we investigated the combined effects of diet and predation on fish gut microbiota.
In our experiment, we fed perch (*Perca fluviatilis*) with three different quantity (5%, 10% and 15% of average fish body weight) levels of chironomidae larvae everyday. Moreover, we exposed perch with/without the presence of pike (*Esox lucius*) to maintain the predation stress for perch. After ten weeks, the whole perch intestine was sampled to evaluate the microbial community composition by sequencing the 16S rRNA gene. We also measured weight, length, intestinal length and sex for all individuals. We sampled the water in order to check the bacterial community from surrounding water in the aquariums.
Our results show that the relative intestine length, which is rather plastic, is shorter in the perch facing predation. While perch fed with higher amount (10% and 15%) of food have longer intestine than the ones with low amount (5%) of food. This strong change in intestine length with treatment suggests that the environment is changed for the gut microbiota too. Thus we expect to see a strong effect of both diet and predation on the microbial community composition.

Our study is therefore one of the first to demonstrate the combined effect of diet and predation risk on gut microbiota in wild animals.

Figure legends
Yellow box is for perch without pike predation, green is with pike predation.
5%, 10%, 15% represent the quantity of food fed to perch

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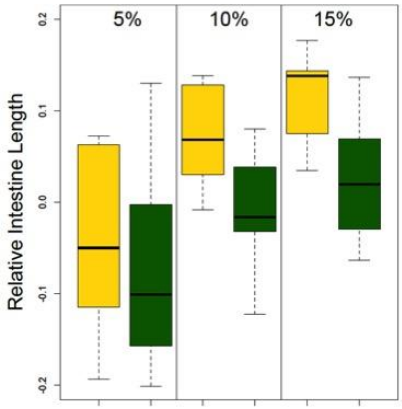
Figure 1



P FOOD 6

From gut to food and back to gut: bacterial diversity in animal casings used in the production of dry fermented sausages

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Introduction: Typical dry fermented sausages have been produced for centuries using natural casings, which are portions of animal intestines derived from slaughtering. Natural casings are very important components of many traditional meat fermented products, and they represent a peculiar case study of gut-derived environments that are used for the production of edible fermented foods.
Materials & Methods: In the present study we investigated by means of culture-dependent methods and Illumina high-throughput sequencing of 16S rRNA amplicons the bacterial ecology of hog, cow and ovine casings at different stages of their preparation for sausages production.
Results: Several strains of *Staphylococcus*, *Lactobacillus*, *Bifidobacterium*, *Vagococcus* and *Clostridium* were counted in significant amounts, isolated and characterized at phylogenetic level. High-throughput sequencing analyses revealed a highly diverse bacterial diversity, which differed strongly between casings of different animal species. The technological processes involved in the preparation for casing had also a strong impact on the casings bacterial ecology, with a significant reduction of undesired microorganisms, and an increase in the proportion of lactobacilli and staphylococci.
Conclusions: Overall results indicate that natural casings are an important source of several bacterial species whose role both in the fermentation and in the microbiological properties of the final products has been underestimated.

P FOOD 7

Influence of food and environmental factors on human gut bacterial community networks in early childhood

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The gut microbiome constitutes a complex ecosystem, where some bacteria compete for nutrition and other bacteria interact with each other to degrade complex food components that cannot be digested by the host itself. Various environmental factors like birth delivery mode, breastfeeding and the introduction of complex solid food components determine the composition of the early gut microbiome during early colonization phases. We intended to describe how functional gut microbial communities in early infancy are influenced by environmental factors by estimating bacterial communities based on interaction networks.
Analysis of microbiota was performed on stool samples from 40 children participating in the German BABYDIET study. Taxonomic identification was obtained from stool samples of children based on Illumina high throughput sequencing of 16S rRNA gene amplicons. In-silico metagenomes were inferred applying PICRUSt .
Our findings demonstrate that the early gut microbiome changes markedly over time.These changes are mainly influenced by the period children were breast fed and by the uptake of solid food components with increasing complexity. A detailed analysis of microbial networks in the gut of babies at 0.5 years of age reveals three bacterial communities which can be distinguished based on their taxonomic and functional composition. We detect a bacterial community in children that are breast fed which is characterized by increased abundances of Bifidobacteriales, Lactobacillales and Enterobacteriales. In contrast, we detect a second microbial community which is dominated by Verrucomicrobiales and Clostridiales where increased abundances of these bacteria are found in children that already received complex solid food components. The third microbial community which shows only low abundances in children born via Caesarean section is dominated by Bacillales and Bacteroidales.
These findings underscore the role of diet and birth delivery mode in the colonization of the early gut microbiome. Comparison of functional traits of the involved bacteria reveals insights into the cross-talk between the gut microbiome and the host metabolism and hints to a functional redundancy for many traits.

P FOOD 8

Fitness landscape of the world's most famous operon in a natural environment

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The lac operon was the first gene regulatory circuit to be clearly understood. For this reason it is widely used as a textbook example to explain how bacteria regulate their metabolic abilities depending on the available resources. It was initially described as essential for the transport and metabolism of lactose in *Escherichia coli* but has since been recognized to be able to degrade other natural substrates as well, such as glycerol galactoside, which is very abundant in most plants. Yet the primary function of the lac operon in a natural environment (the mammalian gut) as well as the selective pressures under which it evolves are still not consensual. *E. coli* is a typical commensal species of the human microbiota and colonizes the gut within hours after birth. About 80% of E.coli isolates carry the lac operon. How important is its presence for the success of *E. coli*'s colonization of the gut and how this affects its interaction with other lactose consumers (such as lactic bacteria) is not fully understood. Here we test the competitive ability of two strains of *E. coli*, that differ in their ability to metabolize lactose, in mouse gut colonization. We do this under two different diet regimes, either supplementing or not the food with lactose. In a diet with lactose we a) observe the emergence of substantial colony morphology variation in the evolving population of *E. coli.* b) determine the genetic basis of this evolved phenotypes and c) test their influence on the microbiota composition under long term consumption of lactose.

P FOOD 9

Unraveling autism: a crowdsourced clinical trial to genotype and sequence the microbiome of autistic children and their siblings

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The existence of a link between the gut and autism is well established, yet mechanisms connecting the two remain poorly understood (1) (2). A complete mapping of the microbial diversity across the autism spectrum could result in dramatic clinical and therapeutic advances. We have initiated a plan to sequence and analyze the gut microbiome from 500 children with autism and one of their sibling within 2 year of age in regard to the genotype of of each of the participants. Leveraging social networks, our goal is to conduct a completely crowdsourced clinical trial that will enroll thousands of internet-active families with autism quickly - in months instead of years. Patients will submit noninvasively collected samples from home, giving us unprecedentedly broad 16S amplicon data and allowing us to search for a microbial community specific to ASD patients. In addition to looking at the microbial structure of the samples, deep metagenome sequencing (>10 million reads per sample) will be performed in a subset of 100 samples. This study aims to improve our understanding of the link between microbiome functionality, genome variation, and ASD phenotype, and reveal the specific mechanisms by which the gut microbiome interacts with autism-related alleles to produce and modify ASD.

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P FOOD 10

Traditional Italian dairy products, a flavorful source of naturally occurring bacteria with beneficial effects on health

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Introduction. Traditional dairy products manufactured with natural starter cultures are generally accepted by consumers for their high nutritional and sensory quality. However, little is known on the role of these products in supplying microorganisms able to stably colonize the human gut and exert probiotic effects.
Objectives. In this study we evaluated the role of a fresh Pasta Filata cheese of the Alto Molise area called "Stracciata", made from milk of cows fed only by grazing and with local dry forages, as a source of probiotic microorganisms.
Materials & methods. The dominant strains belonging to bacterial groups known to comprise probiotic strains, i.e. lactic acid bacteria (LAB) and propionic acid bacteria (PAB) present in the cheese from a single producer were characterized at the genotype level by Rep-PCR and identified by sequencing of the 16S rRNA gene at different times during one week.
The ability of the microorganisms supplied with Stracciata cheese to stably colonize the human gut was evaluated by typing isolates from the faeces of 20 healthy 3 - 6 year old children, who consumed the product for one week, at the end of consumption and after seven and fifteen days from suspension. Multilocus Sequence Typing (MLST) was used to compare the isolates from cheese and from faeces. The ability of bacterial isolates to stimulate IL-10 and TNF-a production by peripheral blood mononuclear cells (PBMC) was determined by commercial kits.
Results. Three *Lactobacillus casei*, one *L. plantarum* and one *Propionibacterium jensenii* with genotypes identical to those found in cheese were isolated from the faeces of some individuals at all sampling times. One *L. casei* strain significantly stimulated the production of anti-inflammatory cytokines *in vitro*.
Conclusions. Results encourage further studies on specifically adapted probiotic bacteria able to confer functional properties to traditional dairy products.

P FOOD 11

Study on the effect of piliation on the colonisation ability of *Lactobacillus rhamnosus* GG

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Among the lactic acid bacteria, *Lactobacillus rhamnosus* GG (Lr GG) is one of the best studied ones and it has a long history of being used in dairy products as a health-promoting bacterium (1, 2). Recent comparative and functional genome analyses showed that Lr GG harbours a gene cluster encoding the host-interacting pili which decorate its cell surface (3). The pili are composed of three pilin monomers: SpaA forms the pilus shaft, while mucus-adherent SpaC and pilus-synthesis terminating SpaB decorate the shaft (4). The sortase C transpeptidase assembles the pili while the sortase A transpeptidase attaches mature pili into the cell wall (5). Previously we described a method in which random mutagenesis was used in combination with a newly developed enrichment method and next generation sequencing to obtain and chatacterise pilus-deficient derivatives of Lr GG (6). In the current study we present the results of a human intervention trial in which we studied the role of piliation on the colonisation ability of Lr GG by using the wild-type strain and its isogenic pilus-less derivative Lr GG-PB12. Strain Lr GG-PB12 chosen for the trial to be used as a control is pilus-less because of a mutation in the gene of sortase C, the enzyme that assembles pili.
The trial was reviewed and accepted by the HUCH ethics committee (HUS 245/13/03/00/13). The trial consisted of a 4-week run-in period, a 2-week intervention period, during which the bacteria were taken daily at a dose of $6x10^{10}$ CFUs, and a 3-week follow-up without consumption. A health and diet questionnaire was filled in by the participants in the beginning and in the end of the trial. The participants were divided in three groups of 9, so that one group consumed Lr GG, the other consumed Lr GG-PB12 and the third group consumed both strains in equal amounts. During the intervention and the follow-up total of 7 faecal samples were collected from all 27 participants. The faeces samples were analysed using quantitative-PCR and cultivation methods to determine the amount of Lr GG strains in the different stages of the trial. The results of the analyses will be presented.

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P FOOD 12

Effects of disodium fumarate on ruminal bacterial communities of early weaning lambs

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Question:Effect of disodium fumarate (DF) on rumnial communities of early weaning lambs is unclear.
Methods: Thirty 50±5 days old male crossbreed lambs of Suffolk and German Merino sheep were used as experimental animals allocated to three groups, 10 lambs each group. The lambs were fed with basic diet and the experimental lambs were fed with basic diet containing 0.5% and 1% of DF for 70 days, respectively. On 70 days of experiment, 4 lambs were selected to be slaughtered each group and the ruminal contents were sampled for extraction of total DNA. The above DNA were used to evaluate rumen communities of lambs by pyrosequencing.
Results: Pyrosequencing analysis: The number of trimmed sequences, Observed OTUs, Diversity-Shannon, ACE, Chao1 and coverage in both experimental groups were not changed significantly Compared with control group. But that of 1% group increased than control group by 5.24%, 9.86%, 4.95%, 4.09% and 8.18% in 1% experimental group.
In total, 24,590 reads were obtained for the 16S rRNA genes by pyrosequencing in the current study. Firmicates, Bacteroidetes, Euryarchaeota, Lentisphaerae were the most important groups and accounted for 92.59% of the reads. At the genus level, the sequences were assigned to 120 different genera(Fig.1). These sequences were mainly related to the genera *Prevotella*, unclassified *Tenericates*, unclassified *Firmicates*, unclassified *Bacteroidetes*, and unclassified

lentisohaerae. The most remarkable change of genera was percentage of *Firmicates* in 1% group compared with control group. Then *Bacteroidetes* ratio to all genera in 1% group decreased than that of control group.
Comparison of the bacterial communities by PCA showed that lambs fed the 1% 0f FD were separated from those receiving control and medium dosage of FD . In general, principal coordinate analysis with unweighted Unifrac showed that PCA axis1 was 34.6% of the variation and PCA axis2 was 27.3% of the variation(Fig.2).
Conclusion: As mentioned earlier, bacterial communities is an important tissue in weaning ruminants. In the present study, early weaning lambs of sheep were used to define the effect of DF on the bacterial communities of rumen. The variation of ruminal communities were found by genus percentage, communities and bacterial diversities.

Figure 1

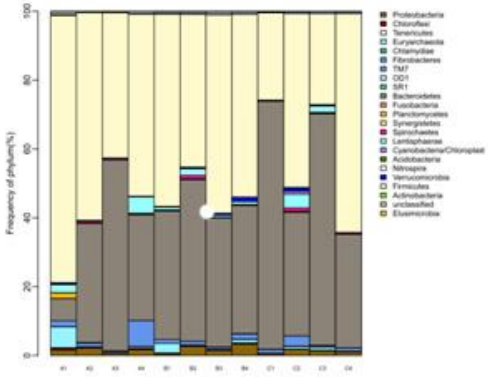
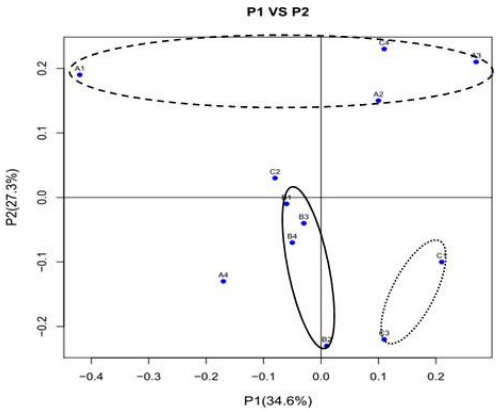


Figure 2



P LATE 1

Differential physiological profiles at the soil particle size level explain the differences between no-till and conventional tillage agricultural managments of the same soil

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A long-term trial (27years-old at time of this sampling) comparing no till *vs.* conventional tillage was settled in a productive agricultural field (38º 07' 10.44" S; 62º 02' 23.50" W) in the south east of Argentinean Pampas (typic argiudoll soil). Tillage or no-tillage was the only difference between two joint plots of 6 Has each, everything else, crops, rotation, agrochemicals, everything else was similar between the two plots. Physical and chemical characterization of those plots show slight differences at the organic matter, water content and density levels. The activities of 5 enzymes clearly differentiate both soil managements both at 0-5 cm and 5-10 cm depth. Also did the measurements of physiological profiles at the community level measured by characterization of the oxygen consumption response after addition of 4 different C sources. Wet sieving fractionation (>63 mm, 63mm<x<20 mm, 20mm<x<2 mm, and 2mm>x) of the 0-5 cm soils from the different managements show different soil particle distribution. DNA extraction from complete sample and DNA extracted from each fraction show quantitative addition result considering the distribution of size fraction for by CLPP for each soil management. Maximum Oxygen consumption for each C source analysed show also additive data between fractions and total soil sample. qPCR quantification of Bacteria and Fungi from total soil and different soil size particle at different soil size fraction show differential distribution of microbial groups. Different soil size fractions show particular physiological profiles despite the soil management of the origin of the particles. This observation suggests that global soil managements differential physiological response is a matter of different quantities of functional microbial communities contributed by the different soil size particle fractions in the particular distribution of soil size fractions as the result of the different agricultural management, conventional tillage or no-tillage. Further quantitative analyses considering particular bacterial groups and bacterial diversity communities will contribute to get a clearer picture and interpretation of soil functioning and the microbes involved in it.

P LATE 2

Situation of genes involved in pathogenicity of potato raised and deep pitted inducing scab bacterium

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Well known from the literature that soil habitant *Streptomyces* group species cause raised and deep pitted lesion on potato which named scab disease. *Nec1* gene and thaxtomine biosynthesis genes including *txtA, txtB, txtC* and *txtD* are the main genes involved in the pathogenicity of these species. Affected potato samples collected from the main potato growing area in Hamedan province and the causative agent *Streptomyces* strains were isolated. Selected strains were examined for the presence and situation of the pathogenicity related genes as they induced variable disease symptoms including raised, netted, shallow and deep pitted lesion on potato tubers under field and green house condition. Pulsed field gel electrophoresis technique revealed that most of the tested strains carried a linear plasmid. Amplification of the pathogenicity genes fragments and southern hybridization analysis showed that only some tested trains harbor *nec1* and *txt* genes. Results of hybridization indicated that only raised and netted scab-inducing strains hybridized to *nec1* and *txt*gen probs. Potato deep pitted inducing strains did not hybridized to above genes probes and did not produced thaxtomin examined by thin layer chromatography (TLC).

P LATE 3

Drug Metabolism of Human Gut Bacteria

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For most current therapeutic drugs it is unknown if and how they are affected by the human gut microbiota [1]. Even for known examples the specifics of when, where, and how are often unclear. However, the general metabolic processes a xenobiotic compound can potentially undergo in the gut are known in principle [2]. To fully understand and predict drug efficacy or potential toxic side effects the impact of bacterial-driven xenobiotic metabolism on the absorption and degradation of therapeutic drugs has to be investigated. To our knowledge, this has never been approached in a systematic manner. To

address this gap, we recently screened a number of potential drug-bacterial interactions relevant to the human gut microbiota. We assessed the decrease in drug concentration in the presence of anaerobic bacterial growth using liquid chromatography. Our main goal was to assess how wide-spread bacterial drug degradation is across therapeutic drugs or across the diversity of gut bacteria.
We found that certain classes of drugs are more likely to be degraded than others. Furthermore, this degradation is bacterium-specific and not all tested gut bacteria degrade therapeutic drugs. In some cases the therapeutic drugs changed the growth behavior of gut bacteria in monoculture. This indicates that not only are drugs affected by bacterial degradation, but also the drugs in turn could affect the balance in the gut microbiota ecosystem.
Our results show that xenobiotic degradation capabilities are widespread in the human gut microbiota and provide a basis for further research on the effects of gut bacterial drug metabolites.

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P LATE 4

Isolation and proteogenomic analysis of a *Sphingomonas haloaromaticamans* strain able to degrade the fungicide *ortho*-phenylphenol used in the fruit-packaging industry

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Question: *Ortho*-phenyl-phenol (OPP) is a fungicide used to prevent fungal infestations of fruits during storage. No methods for the depuration of the wastewater produced by is application are in place, leading to high environmental risks. We aimed to isolate and characterize OPP-degrading bacteria to be used for the biodepuration of those effluents.
Methods: An OPP-contaminated soil was used as a source of microorganisms. Enrichment cultures in minimal medium were followed. Selection on genus-specific agar plates and treatment with different antibiotics were used to purify the OPP degrading bacterium. Analytical methods were followed to characterize the degradation capacity of the isolate in various conditions. A proteogenomic approach was used to elucidate the metabolic pathway and the genes/enzymes involved in it.
Results: A *Sphingomonas haloaromaticamans* strain was isolated and it was able to rapidly degrade up to 150 mg L^{-1} of OPP (as C source). Genome analysis showed the presence of four catabolic operons: an OPP-operon with genes responsible for the transformation of OPP to benzoate and 2-hydroxypenta-2,4-dienoate, two *ben/cat* and a *bph* operon which convert the above intermediates to Krebs cycle intermediates. Degradation of OPP proceeded via formation of 2,3-dihydroxybiphenyl, benzoate, and catechol which were further degraded. Proteomic analysis revealed over 200 genes differentially regulated in the presence of OPP, benzoic acid or succinate. In particular, a monoxygenase present in the OPP operon was >50 up-regulated in the presence of OPP compared to succinate suggesting its involvement in the initial step of OPP transformation. RT-q-PCR for the identified genes is on-going to further confirm their catabolic role.
Conclusions: An OPP-degrading *Sphingomonas haloaromaticamans* strain was isolated and its catabolic capacities were elucidated. The bacterium resulted extremely promising for future application in biodepuration systems.

P LATE 5

Bacterial diversity in sugarcane filter cake following incomplete composting process along 60 days.

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More and more agro industrial residues are being generated and becoming matter of health, social, and environmental public concerns. The organic residues produced by sucroenergetic industry in Brazil are mostly filter cake, bagasse, and vinasse which in turn can be used in soil fertilization for sugarcane crop itself production. Filter cake is rich in phosphorus, potassium, and nitrogen, besides its benefits of moisture through soil coverage. There are recommendations to apply annually this residue into soil as fertilizer, while an excess of it can accumulate. In addition, part of that residue suffers the process of composting, which consist in the bioconversion of agricultural or urbans wastes based on an aeration rate of Carbon:

Nitrogen (30:1) and humidity 60 - 70%. If the complete process is not accomplished following above recommendations, an incomplete composting is obtained presenting less nutrients. We intended to reveal the microbial diversity present in incomplete filter cake composting and understand its partial composting process and its potential as soil manure. For these purposes, we collected 0-20 cm depth of fibrous material onto soil under sugarcane crop to perform DNA extractions and whole genome sequencing by Illumina HiScanSQ (2 x 100). Analyses of unassembled data were performed by MG-RAST using 26,764,894 reads that allowed the identification of three most abundant phyla: Actinobacteria (53.51%), Proteobacteria (25.06%), and Firmicutes (8.60%). Actinobacteria are considered by many studies as bio indicators of soil quality besides their potential as suppliers of antimicrobial peptides, antibiotics, and enzymes that participate on the plant biomass deconstruction (cellusome). Finally, as expected, the composition of that microbiome is very atypical when compared to similar biomes available in IMG/M servers (analysis of the data set assembled) but also there are microorganism commonly found in soils, such as those from Actinomycetales and Rhizobilaes orders.

P LATE 6

Diversity and rehabilitation of prokaryotic and fungal communities of European soil crusts

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Dryland regions cover over 35 % of the Earth´s land mass, including 24 % of Europe. Biological soil crusts (BSCs) provide important ecosystem services such as limitation of soil erosion, retention of water, improving soil fertility, and nitrogen and carbon fixation in these landscapes. The Soil Crust International project (SCIN) in the BiodivERsA programme, was initiated to elaborate strategies for biodiversity conservation and sustainable ecosystem management, since BSCs face high susceptibility of to land use change, livestock grazing, long-term farming and chronic physical disturbance. Soil microorganisms are seldom considered in the context of biodiversity management, even though they essentially contribute to intact ecosystem function. We characterized prokaryotic and fungal communities of BSCs from ecologically distinct dry habitats including mediterranean, nemoral, boreal latitudes, as well as an alpine site. Also, a rehabilitation study is carried out (nemoral and alpine site) to investigate the recovery processes of BSCs in response to anthropogenic perturbation. The successional pattern of recovery is assessed by measuring soil stability, chlorophyll and carbon content and nutrient availability as well as diversity of reappearing organisms including bacteria, fungi, algae, lichens, bryophytes and higher plants. Next-generation sequencing of 16S rRNA gene amplicons showed that Proteobacteria (mainly Alphaproteobacteria), Actinobacteria, Bacteroidetes and Acidobacteria were primarily associated with BSCs. In addition, bacterial communities were distinct depending on soil depth and location. Significant changes were also found after perturbation.

P LATE 7

Land management and seasonal changes impact on chernozems prokaryotic soil microbial community

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Studies of land management influence on soil microorganisms are the current research trend in soil microbiology. The valuable objects of such studies are long-term agricultural experiments on well-investigated soils. In our study a microbiome of Kamennaya Steppe agrochernozem was analyzed by pyrosequencing method.
The aim of this study was an analysis of influence of different land management practices on the taxonomic structure of agrochernozems' prokaryotic biome based on 16s DNA high throughput sequencing data. Seasonal changes were also taken into account.
Our study was conducted on the base of long-term field experiment on the Kamennaya Steppe agroecological station (Voronezh region, Russia). There were plots treated with different doses of mineral fertilizers (NPK). Soil was sampled in 3 replications on 0 - 20 cm depth in May, June and September in 2014 from next plots: a control plot (without fertilization), plots treated by low (30 kg/ha) and high (60 kg/ha) fertilizers doses, fallow plot. Total soil DNA was extracted and 16s DNA sequencing was performed. Data processing was conducted by QIIME program and contained next operations: quality control, sequence library creation, OTU (operational taxonomic units) selection, singletone deletion, taxonomic structure definition, quantification of Shannon index, beta-diversity analysis by UniFrac method.
The dominant prokaryotic phyla in all microbiomes were *Proteobacteria* and *Actinobacteria.* Rates of *Firmicutes* и *Gemmatiomonadetes* phyla were higher in field samples; *Verrucomicrobia* rate was higher in fallow samples. Rates of *Crenarchaeota*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes* и *Verrucomicrobia* phyla were significantly lower in spring samples.

Microbial communities in summer and autumn samples were characterized by high Shannon index. Beta-diversity analysis by UniFrac method showed significant difference in field and fallow microbiome. Different seasons' microbiomes were also separated by this method. No significant difference was found between plots with different fertilization treatment.
Land management and season have a significant influence on taxonomic structure of agrochernozems' prokaryotic biome, but it was not found sensitive to fertilization type.
The work was granted by RSF (grant 14—0079)

P LATE 8
Microbiome taxonomic structure and diversity of two interconnected semi-arid soils of Caspian Depression
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Most of biomolecular analysis of soil microbiomes cover only surface soil horizons. In our opinion, to reveal trends between soil microbiome structure and ecological factors, soil should be considered as a consistent system, so the comparative analysis should cover microbial communities of all soil horizons with contrasting properties.
The aim of this study was comparative analysis of prokaryotic biomes in soil horizons of two contrast soils in semi-arid soil complex.
The objects were two soil profiles of virgin land in northern-west part of Caspian Depression. Soil profile cuts were placed on two opposite micro-relief elements: Episalic Solonetz on high position and Phaeozem in microdepression (20 cm depth). Samples of soil were taken from middle part of every horizon. Then agrochemical soil analysis, total soil DNA extraction and sequencing of 16s RNA gene (GS Junior, Roshe) was conducted. Data was processed with QIIME program.
Diversity of prokaryotic soil microbial community by OTU number, Shannon and Chao1 indexes was lower in deeper horizons in solonetz. Low diversity was also found in solonetz horizon, characterized by high density, heavy granulometric texture, large amount of exchangeable sodium. Just insignificant reduction of biodiversity in lower soil horizons was found in Phaeozem. Families *Enterobacteriaceae*, *Pseudomonadaceae* and *Sphingomonadaceae* were dominant in horizons with low biodiversity. Although upper horizons of Solonetz and Phaeozem had contrast agrochemical properties, the diversity and prokaryotic taxonomic structure were similar. Beta-diversity analysis by UniFrac method showed similarity between upper horizons and increasing difference between lower horizons of two soils.
In authors opinion, the features of this soils microbiomes structure are explained not by difference in main ecological factors (such agrochemical properties as pH, organic content, etc.), but by water regime of studied soils. This conclusion is based on whole soil profile analysis and it couldn't be founded out in case of upper horizons analysis.

P LATE 9
Lichen-associated bacterial communities: studies of bioconversion of lichen metabolites
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The efficiency of antibiotics is worldwide decreasing at a worrying rate. It is therefore most interesting to search for novel active molecules in yet under-explored niches, such as mutualistic microbial symbioses. Lichens are complex organisms, known as a symbiosis between fungus (mycobiont), microalga and/or cyanobacteria (photobionts), and are source of metabolites of interest presenting e.g. antioxidant, antiseptic and antiproliferative activities[1,2]. Lichens also harbor bacterial communities and can be considered as a mini-ecosystem. The abundance and the diversity of the culturable bacterial communities associated with four lichens (*Lichina pygmaea*, *L. confinis*, *Roccella fuciformis* and *Collema auriforme*) were studied: 247 cultivable bacteria strains were isolated and identified by 16S rRNA gene analysis[3]. Associated bacteria are known to produce compounds of interest[5,6], and active compounds (e.g. dicetopiperazines, phenoxazine derivatives, usnic acid) were also isolated from *Streptomyces cyaneofuscatus* strain MOLA 1488 associated with *L. confinis*[4]. To explore potential interactions between lichens and their associated bacterial communities, experiments using lichen extracts and bacteria were performed. The four most abundant bacterial strains associated with *Roccella fuciformis* were chosen to (**1**)

assess the impact of four major metabolites (erythrin, lepraric acid, erythritol and acetylportentol) of this lichen species on the these four bacterial strains by an optimized method of viability using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and also to evaluate (**2**) the ability to bioconversion of these four stains of the chromone lepraric acid and the depside erythrin. These bacteria have shown the ability to metabolize erythrin in orsellinic acid, but none of the four metabolites tested has affected their growth. Orsellinic acid is the biosynthetic precursor of various lichen metabolites (such as usnic acid or depsides, depsidones).These preliminary results suggest potential communication between the partners but further studies are needed for a better understanding of this complex ecosystem.

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P LATE 10

The crystal structure of phthalate dioxygenase reductase protein from a typical ultramicrobacteria PAE-UM

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Ultramicrobacteria are widely distributed in various environments and are of great ecological importance. It is reported that ultramicrobacteria had great potential in environmental remediation. In the current study, one untramicrobacterial strain PAE-UM was isolated from the river sediment. The whole genome of strain PAE-UM was sequenced and the strain was identified as *Curvibacter* sp. Strain PAE-UM can utilize phthalate esters (PAE) as sole carbon and energy source. The results from degradation pathway and genome analysis demonstrated that phthalate dioxygenase reductase (orf 0817) was one of the key enzymes involved in the degradation process. In order to understand the structural basis for its role in degradation, the protein 0817 and the complex of 0817 and NADH were expressed, purified and crystallized. Two coloured crystals were obtained from a recombinant preparation of 0817 overexpressed in *Escherichia coli.* Diffraction data were collected at the Shanghai Synchrotron Radiation Facility to 1.5 Å resolution. We applied the method of crystallography to better understand the degradation mechanism from molecular level. The three-dimension of these two crystal structure were revealed the activity site of the phthalate dioxygenase reductase. The results provide a new perspectives to interpret the pathway of degrading the PAEs and lay a foundation for further study on toxic compounds degradation by ultramicrobacteria in aquatic environment.

P LATE 11

Comparison of bacterial community diversity in sugarcane vinasse

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Question: Production of one liter of sugarcane bioethanol results in 10 liters of waste vinasse. To avoid the high cost of transporting large volumes of vinasse, normal vinasse (NV) is evaporated to obtain concentrated vinasse (CV). NV and CV fertilization has raised concerns regarding greenhouse gas emissions such as N_2O (1). Vinasse microbes may play a major role (2). To characterize a sugarcane vinasse, we combined culturing with gene surveys.
Methods: NV and CV samples were collected with four replicates. Extracts of NV and CV were plated on MRS medium. Physiological tests and amplification of the genes *nifH*, *amoA*, *nirK*, *nirS*, *narG*, *norB* and *nosZ* were carried out for each isolate. The 16S rDNA gene marker of the community DNA was sequenced using the Ion Torrent platform. Sequence processing was accomplished using MOTHUR (3). Data was analysed in R (4).
Results: Six strains of the Bacilli class were isolated from the normal vinasse. The *Lysinibacillus* and *Staphylococcus* strains were positive for the nirK gene. The alpha diversity was lower in CV (H'avg = 1.0) compared with NV (H'avg=2.3). The

phylum represented in the NV and CV samples was *Firmicutes.* The *Lactobacillus* genus made up more than 98% of the CV sample sequences, while in the NV samples, more than 95% of sequences were of the *Lactobacillus*, *Megasphaera* and *Mitsuokella* genuses.

Figure 1. Rarefaction curves of the normal vinasse (green) and concentrated vinasse (blue) samples.

Discussion: Our isolates are not active N cyclers. Further screening with different media may find otherwise. The evaporation procedure from NV to CV appears to select a subset of the NV community, which may have different effects in field fertilization with NV *versus* CV.

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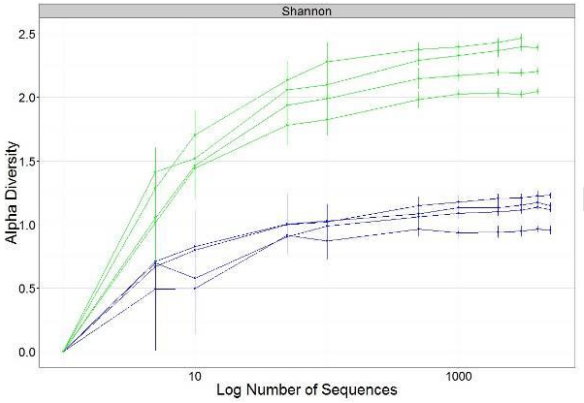
Figure 1



P LATE 12

Directional and non-directional β-diversity of fungi along the alpine landscape

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Understanding the biodiversity organization through space has a long-standing interest in ecology and is essential for predicting ecosystem functioning. In terrestrial ecosystems, there is increasing awareness of spatial patterns of microorganisms along large geographical distances. Surprisingly, the few β-diversity studies in microorganisms have not distinguished the turnover along environmental gradient versus non-directional variation in community structure at local and regional scale. Here, we used the relative abundance of soil fungal operational taxonomic units (OTUs) obtained from 103 randomly stratified sites and distributed between 700 to 3000 m in the western Swiss Alps, to investigate whether soil fungal diversity follows directional turnover or non-directional variation. Using the distance-decay of Bray-Curtis similarity, we found a significant directional turnover along the elevational gradient. As well, using non-metric dimensional scaling (nMDS)

analysis, we found significant non-directional variation patterns associated to soil and climate factors. Variation in fungal community structure was characterized by a shift in the relative abundance of widely distributed fungal families as a function of elevation, although some fungi occurred preferentially only at low or high elevations. We conclude that distance and non-distance dependent β-diversity patterns of soil fungi can occur even in high heterogeneity environments such as the Alpine landscape. These results highlight the importance of distinguish both β-diversity types for understanding the variation on microbial community structure.

P LATE 13

Bacteriophages as natural evolutionary enhancers in bacteria.

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Microorganisms together with their predators, phages, constitute a large part of the human-associated eco-systems. Their arms race affects the evolutionary processes both in phages and microbes.
We have studied evolutionary interactions within bacterial - bacteriophage system consisting of a human pathogenic bacteria *Yersinia enterocolitica* and its natural predators, phages, isolated from the sewage waters of Munich city. Initial bacterial contacts with the phages resulted in rapid development of phage resistance by bacteria. However, it was possible to overcome this insusceptibility by isolating new phages active against phage resistant *Yersinia* using the same waist waters stocks. Such serial phage - microbe co-cultivation experiments were repeated several times to enlarge the scope of possible interactions. Whole genome sequencing of both phage resistant bacteria and their cognate predators, demonstrated high similarity of the isolated phages with the *Yersinia*-specific phages but also multiple genetic rearrangements (mostly nucleotide substitutions) in bacterial genomes. All phage isolated were of the T7-like phage group. However, they demonstrated only moderate genome wide similarities to each other. Strong association between the genetic patterns of the spike fibers and specific adsorption makes it possible to predict bacterial sensitivity to other phages possessing the same pattern sequences in the fiber regions.
In turn, a bacterial - phage contact results in multiple nucleotide sequence alterations in bacterial genomes that might reflect a SOS mediated response of the prey to the predator's attack. This leads to a serious increase in mutation rates that might result in unwanted output, e.g. resistance to antibiotics. We found *Yersinia* clone that has acquired a mutation in the *gyrB* gene and developed a phenotypical resistance to antibiotic ciprofloxacin.
Thus stress imposed by a phage attack on pathogenic bacteria might seriously increase their ability to evolve rapidly and develop numerous unwanted characters, like resistance to common used antibiotics.

P LATE 14

Comparing the Gut Microbiota of Two Communities from Amazon Region with the Southeastern in Rio de Janeiro, Brazil

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The human gastrointestinal tract contains all three domains of life, bacteria, archaea, and eukarya. All the bacteria that reside in the human gut achieve cell densities higher than any other ecosystem. The human gut microbiota plays a main role in human health by acting as a barrier against pathogens and exerting important metabolic and immunologic functions. Studies about the human gut microbiota under different dietary habits and sanitary conditions can provide new perspectives. Hence, the aim this present work was to characterize the profile of intestinal biota colonization of individuals belonging to two different communities in the Amazon region, in Brazil and compare that profile with individuals from the southeastern of Brazil and try to correlate any difference found with their food source habits. To achieve these goals, 68 stool samples were collected. Twenty-eight from each community, Puruzinho Lake and Buiuçu, both on the waterfront Madeira River in the neighborhood of Humaita, all located in the Amazon state; and 12 in the Rio de Janeiro state. To perform a comparative analysis of intestinal microbiota, total DNA was isolated using QIAamp DNA Stool Mini kit and amplified by PCR. A pair of primers targeting the 16S rDNA V3 region was used. For all samples, an amplicon corresponding to 236 pb was obtained. To access the intestinal microbiota composition, a denaturing gradient gel electrophoresis (DGGE) was applied, which is a molecular biology tool widely used to characterize bacterial ecosystems. The DGGE revealed a multi-band fingerprinting in

all samples analyzed. Moreover, preliminary results revealed DGGE fingerprint homogeneous among individuals from both Amazon communities. However, when compared with samples of individuals from southeastern of Brazil this profile was slightly different. Our preliminary results were able to conclude that, differences in gut microbiota of people belonging to those two different Brazilian regions may be due to different eating habits. So, our results encourage us to continue our studies and find out if the gut microbiome of these individuals might be influenced by their food source or/and way of living.

P LATE 15

Biodegradation of selected plastics by psammon bacteria occupying the sand of a recreational marine beach.

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Introduction: In the recent years different types of polymers constitute the significant part among the organic matter accumulated in the sand of sea beaches. The number of polymers has been dynamically growing and it can be even seen in some of the remotest places on earth. Only some polymers are biodegradable and can be transformed for composting. Bacterial biofilms of complex structure and taxonomic composition constitute the key role in the process of their depolymerisation.
Objectives: The aim is to investigate whether the bacteria depolymerise plastic and taxonomic groups involved in this process.
Materials & methods The research was carried concerning the biodegradation of selected plastics by psammon occupying the sand of the sea beach. The research was carried on the sandy sea beach in the central coast of the Baltic Sea. 240 bacterial strains were isolated from the sand, which were tested in terms of their ability for depolymerisation of foil strips produced from: PVA, cellulose acetate and biodegradable thermoplastic starch. Simultaneously the research was carried in situ which tested decomposition of foil strips buried in the sand. The following was tested: the degree of foil degradation, the intensity of bacterial biofilm development on the surface of the foil and the taxonomic diversity using the in situ hybridization (FISH) method.
Results: It was stated that only 2.5% bacterial strains performed the clear biodegradation of poly starch foil and cellulose acetate foil. PVA foil was far more susceptible to biodegradation**.** Over 15% isolated strains intensively decomposed the mentioned plastics. In the research of foil stripe biodegradation on the beach in situ it was observed that poly starch foil is more intensely depolymerized by the bacterial biofilm than cellulose acetate foil. The research of biofilm taxonomic composition showed that in case of poly starch foil *γ-proteobacteria* and *Flavobacterium* were dominant, while besides *γ-proteobacteria* and *Pseudomonas* was numerous on cellulose acetate foils.
Conclusion: Thermoplastic starch in the ecotone of the sea beach is easily colonized by bacterial biofilm, but the rate of decomposition is not much higher than cellulose acetate.

P LATE 16

Biogeography of soil nitrogen fixing bacteria in the western Swiss Alps

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The Alps represent an important opportunity for biogeographical studies thanks to their heterogeneous topography and broad range of different climate and physico-chemical conditions along a wide elevation gradient. The western Swiss Alps region of canton Vaud has been the subject of a long standing series of studies by the University of Lausanne that has investigated the ecology of plants and insects as a function of climate and land use change. More recently focus has also shifted toward the biogeography of alpine microorganisms in this same study area (refer to poster by Yashiro *et al.*). In this project the goal was to characterize the bacteria involved in nitrogen cycling in alpine soils. The nitrogen cycle is one of the most important biogechemical processes in terrestrial ecosystems. It includes nitrogen fixation, mineralization, nitrification and denitrification. Bacteria and Archaea that possess the enzyme nitrogenase are responsible for the fixation of atmospheric nitrogen into more bioavailable ammonia. Among the different genes that encode for the multiple subunits of the nitrogenase enzyme complex, the *nifH* gene is the most sequenced and has become a standard marker gene for styding the ecology of the nitrogen-fixing microorganisms in various ecosystems. Here we present the methods used for amplifying and

sequencing the nitrogenase gene from the alpine soil metagenome and first results on the distribution and ecology of the nitrogen fixing bacteria along an elevation gradient of 700 to 3000 m across the alpine landscape.

P LATE 17

The Transferome analysis allows the inference of the current and paleo-environments

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Introduction: Horizontal Gene Transfers (HGT) are nowadays considered as a major driving force of innovation and evolution in genomes. It was already proved that the frequency of HGT depends on genomic and physiological features (internal constraints) of transfer partners. These features are usually similar between bacteria from same phyla. Thus, the phylogenetic proximity has probably an impact on HGT. Various stress conditions (external constraints) may also modulate rates of HGT. The environment (external constraint) is also supposed to play a key role in HGT, it influences it by regulating the quantity ofthepotential partners of transfer. Environment impact on HGT was illustrated in different examples, but a large study on this question has never been realized.
Question: The objective of our work was to define the real importance of environmental impact on HorizontalGene Transfers, all along the evolution of species.
Methods: We implemented a phylogenomic strategy, in order to detect the HGT that occured during the evolution of each species from six bacterial phyla: *Planctomycetes, Verrucomicrobiae, Chlamydiae, Bacteroidetes, Spirochaetes* and *Chlorobi*. For each HGT detected (ancient and recent), we determined with which organism the transfer was realized. We calculated the percentages of exchanges of each species studied with the different partners. Then, we grouped bacteria according to the environment or the phyla. Thanks to the comparison of variance, we determined whether there is a significant difference of HGT partners between the bacterial groups or not. To finish, weperformeda Principal Components Analysis (PCA) to identify the preferential partners of each species studied and observe the heterogeneity in bacterial groups.
Results: At the end, we can differentiate significantly two main groups of bacteria thanks to the Transferome analysis: Environmental bacteria (bacteria from water and soil) and bacteria living in Vertebrates. These bacteria clearly exchange with different partners. (Environmental bacteria exchange more with *Cyanobacteria* and *Proteobacteria*, whereas bacteria of Vertebrates transfer more with *Firmicutes*)
Moreover, Chlamydia present an important quantity of ancient exchanges with Eukaryotes. The exchanges could be considered as a trace of ancient relation between Chlamydia and Plants.
Conclusion : We propose that Horizontal Genes Transfers could be used to infer the bacterial environment of modern species and also, to reconstruct the paleo-environment of their ancestors.

P LATE 18

Potential of an actinomycete, *Micromonospora halophytica*, to enhance tolerance of tomato (*Lycopersicon esculentum*) to soil salinity through production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase

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The objective of this work was to evaluate whether actinomycetes isolated from saline soils in the United Arab Emirates (UAE) can increase tolerance in tomato plants (*Lycopersicon esculentum*) to salt stress through the reduction in the endogenous levels of the stress hormone ethylene. Thirteen isolates of streptomycete (SA) and non-streptomycete actinomycetes (NSA) that showed ACC deaminase activity were obtained from a total of 45 isolates. DNA analysis of the 16S RNA indicated that the most promising isolate selected produced the highest levels of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase *in vitro* and was identified as *Micromonospora halophytica*. This wild type isolate (WT) significantly increased the fresh and dry weights, and root and shoot lengths of tomato grown under saline condition, both under gnotobiotic and greenhouse conditions. The application of the WT strain of *M. halophytica* significantly, reduced the endogenous levels of ACC, the immediate precursor of the hormone ethylene, in the roots and shoots compared with the non-inoculated control treatment. In comparison, an ACC deaminase non-producing mutant strain (MT) and *Streptomyces atrovirens* which acted as negative controls failed to reduce the endogenous levels of ACC in the roots and shoots and failed to promote plant growth under saline conditions both under gnotobiotic and greenhouse conditions. The application of the WT strain also significantly, increased photosynthetic pigment contents, plant water use efficiency, transpiration rate,

stomatal conductance, and photosynthetic rate, compared with control non-bacterized seedlings. The WT strain and *S. atrovirens* were incapable of producing detectable levels of indole-3-acetic acid (IAA), indole-3-pyruvic acid (IPYA), putrescine (Put), spermidine (Spd), spermine (Spm), gibberellic acid, isopentenyl adenine, isopentenyl adenoside and zeatin in their culture filtrates. The application of the WT and MT strains or *S. atrovirens* failed to increase the *in planta* levels of endogenous plant growth regulators tested including IAA, IPYA, Put, Spd and Spmin the roots and shoots. This study is the first published report to demonstrate the potential of actinomycetes to ameliorate the deleterious effects of salinity stress on plant growth through promotion of plant growth.

P LATE 19

Promotion of growth of *Salicornia bigelovii* in a calcareous saline soil by phosphate-solubilizing halophilic actinomycetes

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Salicornia is an environment friendly annual, leafless, succulent halophytic oil-bearing plant that can be readily grown on untreated seawater which is found along the coastline of many countries. The aim of this study was to determine the potential of halophilic actinomycetes to solubilize insoluble phosphates in soil and to promote *Salicornia* growth. Thirty-nine halophilic actinomycetes were isolated from a calcareous saline soil deficient in available phosphorus (P) in the United Arab Emirates. Twelve of these isolates solubilized powdered rock phosphate (PRP) in solid and liquid media. These isolates solubilized considerable amounts of P, caused a significant drop in pH in a liquid medium amended with PRP, produced alkaline and acid phosphatases, as well as a variety of organic acids. Seven isolates were initially selected based on their exceptional rhizosphere competence. These seven halophilic actinomycetes isolates were chosen because of its strong ability to colonize *Salicornia bigelovii* roots up to a depth of 14 cm. In the greenhouse, the application of these seven isolates to soil amended with either single super-phosphate (SP) or PRP significantly promoted the growth of roots and shoots of *S. bigelovii* plants compared with those of plants grown in non-inoculated soil amended with SP or PRP. This was also evident in the significant increases in the concentration of available P in the soil and in the levels of N, P, K, S, Mg, Fe and Zn in the roots and shoots of inoculated *S. bigelovii* plants. The plant growth promotion by these seven isolates was most pronounced in the presence of SP as soil amendment compared to PRP. This study is the first published report to demonstrate the potential of phosphate-solubilizing halophilic actinomycetes to promote the growth of the halophytic plant *S. bigelovii*. The current study demonstrates the feasibility of using halophilic actinomycetes to promote the growth of *S. bigelovii* cultivated under greenhouse and potentially for field application for forage and seed production in proposed seawater irrigated production of *Salicornia*. This enhancement of growth by actinomycetes in the UAE is expected to increase the large scale production of *Salicornia* biomass that can be utilized not only for feed and culinary purposes but also for large scale production as a source of biofuels.

P LATE 20

Invading and getting invaded: a view from both sides

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The use of microbial communities in water treatment has a long history, but the underlying processes are largely unknown. Additionally, due to the rising demand for safe drinking water, the Urban Water Cycle UWC (water-to-wastewater-to-water) has to be closed in a sustainable and cost-efficient manner. Therefore, the active control of microbial communities and their ecosystems to achieve a desired outcome in water treatment is a challenging task in Microbial Resource Management and Engineering (MRME).

Microbial communities are exposed to fluctuations, e.g., in temperature, nutrients, pH, and microbes that invade or leave the resident community. Our work aims to describe the link between the structure of drinking water-related bacterial communities and invasion capacity by drinking water contaminants, such as *Escherichia coli*. We hypothesize that co-evolution of the microbial community and invasion success are linked. Using microcosms and flow cytometry (FCM) we will monitor the impact of invasion on biodiversity (Illumina sequencing) and functionality in synthetic communities, first under static and later under oscillating temperature conditions.

We screened more than 20 sandfilter isolates for their growth and selected 10 candidates to compose synthetic communities. Currently, invasion assays under static temperature with initially even synthetic communities and a GFP-

expressing *E. coli* invader strain are performed. During co-evolution of the community over four generations, every generation is challenged with the invader. Invasion is then determined by turbidity measurement (OD_{600}) and FCM for both total cell count and GFP-labeled cell counts.
We found that during co-evolution of the microbial community the percentage of invasion increased, and the invader had a negative effect on the growth of the community. Assuming that a shift in community evenness occurs over time, this observation is consistent with previous findings that uneven communities are easier invaded (1).
This indicates that the implementation of MRME in drinking water treatment through controlling the evenness of microbial communities with view to invasion by waterborne pathogens, might facilitate the overall goal to provide safe drinking water and to close the UWC.
(1) de Roy et al., 2013. Nature Communications 4: 1383

P LATE 21
Identification and genome analysis of a novel *Pseudomonas veronii* isolate with biocontrol activity against the nematode *Xiphinema index*
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Pseudomonas sp. R4 is a novel ɣ-proteobacterium isolated from a vine root that exhibited secondary root inducing activity and antagonistic activity against the nematode *Xiphinema index*. This nematode reduces the fitness of grapevines altering theuptake ofwater, nutrient and transmitting important viruses associated to fanleaf degeneration.The aim of this study is to determine the gene clusters of strain R4 involved in the metabolism of phytohormones and determining the molecular basis of nematicide biocontrol activity. The genome of isolate R4 was sequenced using 454 pyrosequencing technologies and subsequently assembled. A phylogenetic tree was built based on the concatenated alignment of 31 universal protein orthologues. *Pseudomonas* sp. R4 showed the closest relationship with *P. veronii* 1YdBTEX2. Comparative analysis of strain R4 and *Pseudomonas* spp. genomes allowed the identification of gene clusters that encode complete synthetic pathways for plant growth promoting compounds (indole-3-acetic acid, acetoin and butanediol) and phytohormones catabolism pathways(ethylene and ɣ-aminobutyric acid) secreted by plant in response to stress. Extracellular proteases, lipases and different secretion systems (T1SS, T2SS and T3SS) involved in the transportation of exoenzymes are encoded in the R4 genome. Genes encoding an elastase and phospholipase showed high identity to functional LasB and ExoU from *P. aeruginosa*, respectively. The latter enzymeis avirulence factor associated to T3SS. Lipase activity assays on tributyrin-agar Petri dishes demonstrated that R4 bacterial cultures synthesized exolipases. Cell supernatants obtained from milk induction assays showed preliminary exolipase activity in tributyrin agar. Zymograms revealed activities in strain R4 supernatants. Screening performed on gelatin Petri dishes showedthat R4 produced protease degradation halos. Zymograms using the extracellular fraction revealed the induction of three proteases.These results showed a stronger secretable enzymatic activity suggesting that this activity in strain R4 is probablyinvolved in the antagonistic activity of this isolate against *X. index*. Moreover, strain R4 could influence plant growth and development by producing or degrading plant hormones that modulate plant regulatory mechanisms.

P LATE 22
Effect of the soil types and the plant species on the rhizosphere competence and biocontrol activity of *Pseudomonas jessenii* RU47 at the field scale
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Soil-borne pathogens are responsible for serial crop losses worldwide and hard to control. It is well documented that the treatment of plants with bacteria living in association with the plant are an environmental friendly control method against soil-

borne pathogens such as *Rhizoctonia solani*. The effect of an inoculant strains are inconsistent at the field scale but the reason for this variability is largely unexplored. Hence, an improved understanding of factors influencing the biocontrol activity of bacterial inoculants is needed. We assume that both the plant species (lettuce, potato) and the soil type can affect the ability of a bacterial inoculant to colonize the rhizosphere in a sufficient density and its biocontrol effect against soil-borne diseases, respectively. An experimental field plot system with three different soil types enabled us to investigate the effect of both factors on the rhizosphere competence of the inoculant strain *Pseudomonas jessenii* RU47 and its biocontrol efficacy. The soils of the experimental plot system shared for more than ten years similar agricultural management strategies. The inoculant RU47 showed a high the rhizosphere competence in lettuce and potato during the whole growth period with significant control effects against *R. solani*. The rhizosphere competence of RU47 assessed by selective plating was not affected by the soil type. In addition, the effect of RU47 and the pathogens *R. solani* on the bacterial community composition in the rhizosphere was studied by denaturing gradient gel electrophoresis of 16S rRNA gene fragments amplified from total community DNA. The inoculant RU47 had negligible influence on the bacterial community composition in rhizosphere of potato, but more pronounced effects in the lettuce rhizosphere. The effects of the pathogen *R. solani* on the bacterial community composition in the lettuce rhizosphere were negligible, whereas *R. solani* effects in the potato rhizosphere were more pronounced. These differences were likely caused by the different pathogenesis of the two pathogens.

P LATE 23

Effect of Microbial extracts of Algerian soil against *Culex pipiens* (Diptera: Culicidae)

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The Culicidae family is probably the best known from the insect class and the most feared by parasitic diseases they can inoculate during their blood meal and the nuisance that their presence constitutes. *Culicidae* are considered as a vector of many diseases such as; malaria, yellow fever, dengue, filariasis, West Nile and some encephalitis.
These insects that are controlled by conventional pesticides such as organophosphates or pyrethroids, have developed a resistance against these insecticides, it has also been proved that their use causes various effects on environment, human health and non target organisms.
The biological control remains the best method because it is safe, selective and biodegradable, in this context, the problematic has been posed to evaluate the toxicity of *Bacillus thuringiensis* against the 4th instar larvae of culex mosquitoes.
Indeed, the Algerian soil presents a high microbial diversity; 15 different soil samples have been collected from many locations in the cities of Constantine and Biskra, in order to isolate the strain of *Bacillus thuringiensis.*
*B*ti is considered as an aerobic Gram-positive bacterium, which is capable of producing a range of toxin insecticides against several insects such as *Culex pipiens* mosquitoes.
The obtained results indicated that there is a high sensitivity of the 4th instar larvae of *Culex pipiens* exposed to the strains studied. *B*t showed a high level of activity with 85% of mortality recorded after three day of exposure to concentration 310µg/ml.
Finally, the *B*t isolated from several Algerian soils can be considered like the entomopathogenic fungi against the larvae of Culex mosquitoes.

P LATE 24

Bacterial biodiversity in a chemolytotrophic cave

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Movile Cave is a unique ecosystem isolated from the outside world and based in chemosynthesis, mainly by sulfur and methane oxidation (Sarbu et al. 1996). Earlier studies of its microbial diversity were based in either cultivation dependent methods or in cloning libraries generation (Chen et al. 2009). Both strategies are usually quite limited because of the low percentage of microorganisms that can be cultivated and the usually small sample sizes in clone libraries.
The aim of this study was to deepen the knowledge of the bacterial community in Movile Cave.
Samples were collected and kept refrigerated until processed. DNA was extracted using PowerSoil DNA isolation kit (Mo Bio). High-throughput sequencing was performed on a MiSeq platform. For the library preparation, PCR with bar-coded

primers was performed per triplicate. PCR products were ligated to tagged sequencing adapters using the TruSeq PCR-Free LT Sample preparation Kit (Illumina). Samples were pooled in equimolar concentrations for sequencing. Then, high-throughput sequencing data were analyzed using QIIME software package and grouped into Operational Taxonomic Units (OTUs). For each OTU, a representative sequence was obtained and identified using the BLASTn program.
A total of 14 samples collected from water, floating mat and limestone walls were analyzed. We found the presence of bacteria corresponding to several different genera as well as some unclassified bacteria. All the samples presented many different OUTs and therefore, despite of having been sealed off from the exterior for several million years, we can confirm that Movile Cave presents a quite diverse bacterial community.

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P LATE 25
Succession of bacterial communities in the forefield of a retreating glacier
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Introduction: Retreating glaciers are a phenomenon that is observed in the Alps since the middle of the 19th century. Though signaling and alarming trend of warming in Central Europe, they also offer a unique chance to study the process of colonization of the barren rock with microbes and the formation of soil.
Objectives: We were interested in studying the bacterial communities in this Alpine environment and how their structure and composition changes with time elapsed since deglaciation. Of special interest was to compare the variability of the microbial communities on a small scale with that on a larger scale.
Materials & Methods: In this metagenomics study, we examined two study areas in the lateral moraine of the Weißkugel (Palla Bianca) glacier in the Central Italian Alps. The first study area (termed "far") has been deglaciated 50 years ago ("old soil"), the second (termed "close") five years ago ("young soil"). We randomly chose six sampling sites from the far area and seven from the close area. Using Illumina's MiSeq technology we obtained > 20 million paired end sequences from the variable regions 3 and 4 of the 16S rRNA gene. We used the software suite Qiime to assemble, clean, and align sequences and to ascribe operational taxonomic units (OTUs). The software Explicet and the R-package phyloseq were used to calculate various indices of diversity.
Results: We found that species diversity was higher in the old soils than in the young soils. The composition of bacterial communities is slightly more equilibrated in the old soils. We identified orders, families, and genera that exclusively occurred in young soil, e.g. sphaerobacterales, acidimicrobiaceae, and staphylococcus.
Conclusion: Next generation sequencing lends itself to monitor the succession of bacterial communities in the forefield of a retreating glacier. Some bacterial orders, families and genera appear to be specific to lately deglaciated rock.

P LATE 26
Competence of two *Streptomyces* strains to colonize lettuce seeds, roots and rhizosphere
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Beneficial microorganisms with positive activity on plant growth and health offer an attractive alternative to conventional agriculture. However, the successful application of biological control agents (BCAs) in agriculture has been hindered by insufficient knowledge of mechanisms by which BCAs interact with the host plants and other microorganisms. Colonization of the plant roots and development of rhizosphere competence are widely considered as crucial steps through which BCAs could create useful interactions with plants, and also protect them against soil borne pathogens. Recently, *Streptomyces* species are gaining increased interest as BCAs due to their different mechanisms to inhibit plant pathogens. We evaluated

one of these mechanisms, the abilities of two *Streptomyces* spp. strains, ZEA17I, and SW06W, to colonize lettuce seeds, rhizosphere, and roots. By conjugation, the strains were tagged with enhanced green fluorescent protein and apramycin resistance genes to study the lettuce colonization dynamics in sterilized sand. The preliminary results showed that both strains could successfully colonize the lettuce seeds in a very short time, and then develop rhizosphere competence up to six weeks. Endophytic colonization of the root tissue was also detected after six weeks. The seed, rhizospheric, and endophytic colonization ability of the two *Streptomyces* strains may contribute to their biocontrol activities against lettuce soil borne fungal pathogens.

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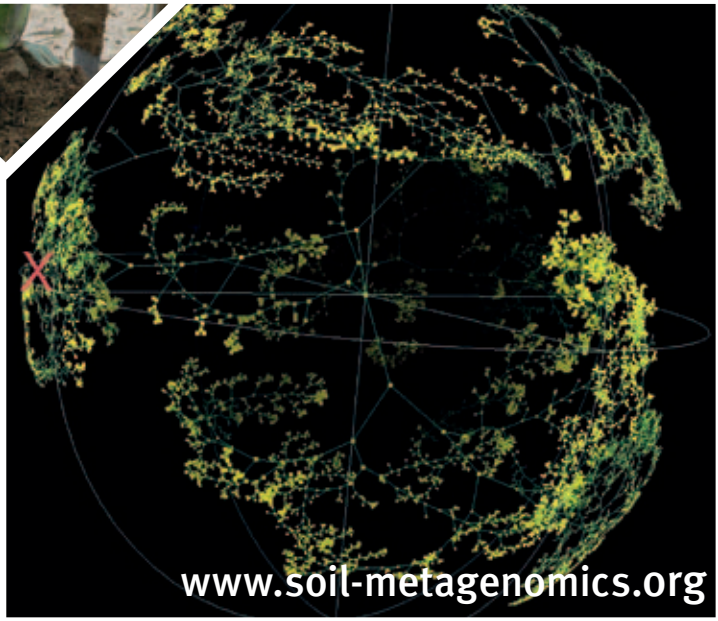

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